Cancer Risk among Italian Veterans from the Balkans: the Activities of the Italian National Institute of Health

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Abstract. In recent years, cases of cancer have been reported among Italian troops involved in the peace-keeping mission in Bosnia and Kosovo. By the end of 2000, the Italian Minister of Defence appointed a Committee of Enquiry to gain a scientifically sound, reliable picture of the health consequences of the exposure of military personnel to Depleted Uranium (DU) and to assess the possible etiologic role DU may have played in the occurrence of specific pathologies. The Committee found a statistically significant excess of Hodgkin's lymphomas, but up to now the lack of thorough knowledge about internal uranium exposure and its potential effects has prevented us from determining whether lymphatic cancers are correlated with DU exposure. The Committee of Enquiry issued several recommendations, in particular: 1) to follow up the cohort of military personnel deployed in Bosnia and Kosovo and monitor the incidence of tumours and the evolution of the ensuing epidemiological scenario, and 2) to list the individuals that could have been exposed to DU for different reasons and enrol them in a long-term programme of medical surveillance. A decision was also made to quantify levels of U and other potentially toxic elements - as well as to measure the isotopic ratio $^{235}U/^{238}U$ for the assessment of DU - in biological samples of soldiers deployed in war theatres where presumably DU weapons were used, together with tentative indexes of contact with possible genotoxic factors. In this paper a general description is given of the activities carried out by the Italian National Institute of Health (ISS, in the Italian acronym) in compliance with both the recommendations of the Committee of Enquiry and the decision to quantify potential exposures.

KEYWORDS: radiation protection, cohort studies, military health, Hodgkin's lymphomas, depleted uranium, potentially toxic elements.

1. Introduction

On December 22nd, 2000 a Committee of Enquiry was appointed by the Italian Minister of Defence on the incidence of malignant neoplasms amongst Italian military personnel deployed in peace-keeping missions in Bosnia and Kosovo. The role of the Committee was to study all medical and scientific aspects of the cases of tumour pathology amongst military personnel, which had been brought to the attention of the Directorate General of Military Health and the media. The Committee also aimed to verify the existence of a correlation with DU weapons used in the area and to identify the various causes of the aforesaid pathologies.

Cancer incidence was calculated in a population of military personnel deployed in Bosnia and/or Kosovo at least once between December 1995 and December 2001. The incidence rates were calculated using the number of cases for each of the pathologies observed as numerator and the total observation period for each subject, expressed in person-years (from the date of initial deployment until December 31st, 2001 or until diagnosis), as denominator. The indicator used in the comparison was the Standardised Incidence Ratio (SIR), i.e. the ratio of tumour cases *observed* in the population of soldiers deployed in Bosnia/Kosovo to *expected* cases amongst the same population, based on the rates in the Italian Cancer Registries. A statistically significant excess of Hodgkin's lymphomas was observed, whereas the total number of solid tumours and malignant neoplasms was significantly lower than expected.

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In the light of the radiation protection discipline, a causal relationship between Hodgkin's disease and internal exposure has not been demonstrated, in the present state of scientific knowledge. The existing studies mostly refer to chronic exposure, under conditions different to those of the soldiers considered here. On the other hand, the same studies make it possible to consider a causal relationship between exposure to uranium in industrial scenarios and excesses of some neoplastic pathologies. We must not forget that what we know about the metabolism of uranium indicates the possibility of development of neoplasms in the lymphatic tissues. Taking the above into due consideration, the excess of Hodgkin's lymphomas seemed therefore worthy of careful analysis, by monitoring the soldiers over a period of time. The Committee of Enquiry also issued several recommendations [1], in particular: 1) to follow up the cohort of the military personnel deployed in Bosnia and Kosovo and monitor the incidence of tumours and the evolution of the ensuing epidemiological situation, and 2) to list the individuals that could have been exposed to DU for different reasons and enrol them in a long-term programme of medical surveillance. A decision was also made to quantify levels of U and possibly of other potentially toxic elements such as As, Cd, Mo, Ni, Pb, V, W and Zr - as well to measure the isotopic ratio $^{235}U/^{238}U$ for the assessment of DU - in biological samples of soldiers deployed in war theatres where DU weapons were presumably used, together with tentative indexes of contact with potentially genotoxic factors.

In this context the Italian National Institute of Health has made two scientific collaboration agreements with the Ministry of Health and one with the Ministry of Defence.

The aim of the first agreement (see paragraph 2) is to carry out comprehensive epidemiological studies on Italian soldiers who participated in peace-keeping missions in Bosnia and Kosovo.

The second agreement (see paragraph 3) focuses on: 1) the analysis and critical revision of the relevant scientific literature, including available epidemiological studies on cancer risk among Gulf war and Balkans veterans, and 2) the development of tools for objective scientific knowledge on DU to be conveyed to the media and the public.

The third agreement (see paragraph 4) regards the measurement of concentrations of U and other potentially toxic elements along with the assessment of DU in urine, serum and hair sampled before and after deployment in Iraq. The analytical approach basically resorts to two particularly fit-for-purpose techniques, namely, Sector Field Inductively Coupled Plasma – Mass Spectrometry (SF-ICP-MS) and Direct Reaction Cell Quadrupole Inductively Coupled Plasma – Mass Spectrometry (DRC-Q-ICP-MS).

2. Epidemiological studies

The epidemiological investigations planned by the Italian National Institute of Health consist in retrospective cohort studies aimed at ascertaining the cause-specific mortality rates and the cancer incidence rates among Italian soldiers who participated in peace-keeping missions in Bosnia-Herzegovina and Kosovo, where DU munitions were used.

The mortality and incidence rates observed in the Bosnia and Kosovo (BK) cohort will be compared to the rates experienced by a cohort of non-deployed soldiers (ND) and to the general population rates. Deployments to Bosnia and Kosovo occurring from 1995 to 2004 will be individually identified from the files of the Defence Health Services. The BK cohort is estimated in about 65,000 members. A comparison group, of the same size and matched to the BK cohort on age and period of service, will be randomly selected among the ND military personnel.

In the context of the mortality cohort study, the vital status of each member of both cohorts will be ascertained through the population registry of the municipality of residence; the date and place of death of deceased subjects will also be retrieved through the Population Offices. Using the information about place and date of birth, place and date of death, and place of residence at death, through a cross-linkage with the National Death Index we will identify the causes of deaths.

Person-years at risk of death will be counted, for each subject, from the date of first deployment to Bosnia or Kosovo to the earliest of the following dates: December 31st, 2004 (if alive at the end of the observation period), date of emigration, death, or lost at follow-up. The expected number of deaths will be calculated by multiplying age-, sex- and period-specific person-years as from the BK cohort by the corresponding cause-specific mortality rates of the Italian population. Cause-specific standardized mortality ratios (SMR) will be calculated as the ratio of observed and expected deaths. Hazard ratios derived from Cox proportional hazard models will be used to compare the mortality experience of the BK and ND cohorts.

As to the cancer incidence cohort study, in want of a national Cancer Registry in Italy (local cancer registries cover around 25% of the Italian population), incident cases of cancer among members of both cohorts will be ascertained by a record-linkage with the national data-base of hospital discharges (SDO, in the Italian acronym), supplemented by an exhaustive review of the clinical records, aimed at verifying the discharge diagnosis recorded in the SDO and at collecting the diagnosis date (ideally the date of the pathology report).

The statistical analysis will parallel that of the mortality study. The expected number of cancers will be calculated by multiplying the age-, sex- and period-specific person years as from the BK cohort by the corresponding neoplasm-specific incidence rates of the Italian population (estimated from the pooled data of the Local Cancer Registries); SIR will be calculated. Hazard ratios derived from Cox proportional hazard models will be used to compare the cancer incidence experience of the BK and ND cohorts.

3. The analysis and critical revision of the relevant literature

The widespread concern regarding the occurrence of disease among military personnel deployed in war theatres or during peacekeeping operations has given rise to numerous publications. In Italy, as mentioned earlier, the hypothesis gained great prominence of a correlation between the incidence of cancers in soldiers deployed in Bosnia and Kosovo and the use of DU munitions in these areas.

Many scientific studies on this issue were published in peer-reviewed papers and reports - generally accessible on the Internet - published by important scientific institutions. Moreover, associations and groups of opinion have produced several documents classifiable as *grey literature*, without considering the amount of articles appeared in the media. The large quantity of documents of this kind was inevitably neglected by researchers and institutions, but has attracted and kept alive the interest of the public, whereas the scientific community has not always given as broad and effective answers through channels with high impact and in a language accessible to the public at large. In the light of these considerations, the Minister of Health asked the ISS for the thorough reading and critical review of scientific work aimed at clarifying the interaction between DU and exposed people: 1) assessment of exposure levels and pathways; 2) description of the main biological and health effects related to dosimetric assessments; 3) determination of risk coefficients. This activity over time will foster the organization of workshops and meetings with the population, in order to communicate updated scientific evidence, using an accessible, technically correct language. In this way the actual state of knowledge in the field can be transferred to the public opinion.

To achieve these objectives the scientific literature directly or indirectly relating to the DU uranium issue has been collected, listed and critically analyzed, a pursuit that is being extended to the *grey literature* and any other documentation of interest. These activities can bring to light completed or ongoing research in the last years which have poor visibility in the scientific context, owing to their small size or limited funding. Indeed, at least in Europe, there is no large research project involving different groups and institutions or drawing on important funding such as EU funds, which can gain adequate resonance and disseminate knowledge. It is worthy of note that in 2001 the EU Commission seemed to be interested in the submission of collaborative projects for the study of health issues associated with DU, following the outcry raised by the first reported cases of cancer among soldiers on mission in the Balkans. At least two projects were submitted - one involving the ISS - but none received funding.

As for the methods we are using to review the literature, they depend on the type of papers published. The review – which is still under way - began by accounting for the existing databases and evaluating the quality of any single paper. About 1100 references have been collected so far, with about 650 peer reviewed papers, 95 reports by international or national institutions, 60 conference proceedings and many others (grey literature). With this rich reference database, a Web site is being constructed, where the public and researchers can find a collection of bibliographic information and abstracts of peer reviewed papers.

This activity will also result in critical revisions to be presented in open workshops, in different peer reviewed papers meant for the scientific community and notes to be published on the Internet for a wider audience. The epidemiological studies of cancer risk among Gulf war and Balkans veterans have been the subject of a recently published review [2]. Some ISS researchers involved in the DU issue [3,4] have already organized a scientific event addressed to both the public and researchers. In October 2004 an International Workshop was held at the ISS to exchange results and reflections, open a debate on such a controversial issue and outline possible collaborations. Experts from various national and international institutions (Universities in Italy and abroad, IAEA, IARC, etc.) were invited to address the various aspects related to the use of DU, specifically measurement techniques, environmental monitoring, biological monitoring of population and soldiers, epidemiology of exposed people, DU-related radiation protection problems, and strategies for public communication. The event was attended by Italian and foreign researchers, representatives of the press and associations interested in the issue. The articulated discussions during the workshop revealed a substantial uncertainty about the role of DU in the aetiology of diseases among potentially exposed people and, once again, the need was stressed to proceed with further research.

Aware of the gap of knowledge which still characterizes the issue, ISS experts have decided to organize a second International Workshop, to be held on December 17th, 2008 in ISS, specifically focusing on updating DU research. Some of the key issues to be addressed will be epidemiological study of exposed military personnel, study on the biological effects of DU, and environmental and biological monitoring. The Workshop will be an opportunity to share new scientific research and engage in dialogue concerning research prospects in this field as well as future activity programs.

4. Determination of trace elements in the framework of the SIGNUM Project

4.1 Scope and experimental design

Late in 2003 the Italian Ministry of Defence launched a project named *Studio dell'Impatto Genotossico Nelle Unità Militari* (SIGNUM, Study of the Genotoxic Impact on Military Units). This project, still in progress, aims at: 1) assessing the actual exposure of military units to DU and other potentially genotoxic chemicals; 2) ascertaining the possible exposure to unforeseen mutagenic or carcinogenic substances; 3) evaluating the risk of tumor pathologies on the basis of the frequency variations in the markers under test.

The following Italian research institutions participate in the SIGNUM Project:

- *National Institute of Health*, Rome;
- *Mendel Institute*, Rome;
- *National Institute on Cancer Research*, Genoa;
- *Study and Research Center on Health and Veterinary Science of the Italian Army*, Rome;
- *University of Rome "Tor Vergata"*;
- *University of Genoa*;
- *University of Pisa*.

The above-mentioned third agreement is part of the SIGNUM project.

Iraq was chosen because, according to official information, a large but unknown amount of DU-containing weapons were used during the last Gulf war of 2002. Moreover, several industrial plants were present in the area of concern, with the ensuing high risk of environmental pollution. This study

is based on the voluntary participation of individuals belonging to the Italian Armed Forces and serving in Iraq on a temporary basis. The population under test was randomized to guarantee the statistical and epidemiological validity of the study. About 1000 individuals were enrolled in the study. This statistical potency is adequate for molecular epidemiology investigations that use biomarkers of genetic damage.

The Project is expected to allow for the assessment of the risk posed by DU to the selected population of *ca.* 1000 individuals as well as for the assessment of the risk posed to subgroups of this population as identified through proper statistical criteria.

The following materials were sampled before deployment in Iraq and at the end of the mission: 1) urine (*ca.* 50 ml per sample); 2) blood (*ca.* 50 ml per sample); 3) hair (*ca*. 300 mg per sample). All samples were assigned a code. The identity of the subjects is kept confidential.

Potentially toxic elements are being determined in urine and serum samples (U, As, Cd, Mo, Ni, Pb, V, W, Zr) along with the isotopic ratio $^{235}U/^{238}U$. An aliquot of each sample is frozen and stored for at least 30 years. If the need arises, these aliquots can be analyzed to confirm previous determinations on the same samples or to perform new tests that the advancement of knowledge may render necessary.

All tools and containers used to sample and store the materials under test are carefully decontaminated as necessary in order to minimize any possible exogenous contribution to the analytes of interest. To this end, talc-free gloves, teflon and high-density polyethylene containers and non-steel scissors are used. Samples are pretreated in a Class 100 Clean Laboratory.

The complexity of the project has entailed the management of organizational and administrative aspects, which included the training of military personnel to accurate collect subjects' personal data, and to properly sample and store the biological matrices.

4.2 The analytical approach

Determinations are being performed with Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS) for isotopic ratios $^{235}U/^{238}U$, and Direct Reaction Cell Quadrupole Inductively Coupled Plasma Mass Spectrometry (DRC-Q-ICP-MS) primarily to quantify the total concentrations of trace elements [5-7]. An Inductively Coupled Plasma (ICP) is a highly ionised inert gas (mostly argon) sustained by a Radio Frequency (RF) field. The high temperatures reached in the plasma successively desolvate, vaporise, excite and ionise atoms from the sample. The plasma is formed by means of a metal coil (the load coil) surrounding connected to an RF generator. Power (mostly 700-1500 W) is applied through the coil and an oscillating magnetic field corresponding to the frequency of the generator is formed. The charged particles (electrons and ions) within the plasma are forced to flow in a closed annular path. As they meet resistance to their flow, heating takes place producing additional ionisation. The process occurs almost instantaneously and the plasma expands to its full strength and dimensions. The ICP appears as an intense, very bright, plume-shaped plasma. At the base the plasma is toroidal (induction region). Samples are introduced through the induction region into the centre of plasma.

Mass spectrometry-based plasma techniques resort to the plasma to generate charged ions from the element species within a sample. These ions are then directed into a mass spectrometer which separates them according to their mass-to-charge (m/z) ratio. Most mass spectrometers with a plasma source have a quadrupole system or a magnetic sector and work at a vacuum of 10^{-8} Pa or less. After passing through the sampler and skimmer cones and the ion optics, ions of the selected mass/charge ratio are directed to the detector, where ion currents are converted into electrical signals. Each element is quantified according to the number of ions arriving and generating electrical pulses per unit time.

Mass interferences are the major problem with such techniques, e.g., by isobaric species which significantly overlap the mass signal of the ions of interest, especially in the central part of the mass

range (i.e., 40-80 amu). Matrix interference may also occur with some analytes. These phenomena may lead to the suppression of the analyte signals.
The prime characteristic of an ICP-MS instrument is its resolution, i.e., the efficiency of separation of two close masses. Quadrupole instruments are in this regard inferior to magnetic-sector spectrometers. The detection power of the two techniques is in the range of μg/L and ng/L, respectively.

The results of the trace-element measurements will be compared with the results of the genotoxicity investigations. The conclusion of the SIGNUM project is foreseen for spring 2009.

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