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Long term mortality and morbidity of Italian soldiers after deployment in Iraq as related to biomarkers assessment: Results of the SIGNUM study

R. De Angelis [a], A. Piciocchi [a], M. Bugani [a], S. Rossi [a], M. Lastilla [b], R. Vento [c], A. Autore [c], N. Sebastiani [d], S. Palomba [d], V. Manno [e], S. Battilomo [f], S. Belmonte [f], A. Pulliero [g], A. Izzotti [h,i,*]

[a] Department of Oncology and Molecular Medicine, Istituto Superiore di Sanita, Rome, Italy
[b] Aerospace Medicine Institute, Italian Air Force, Rome, Italy
[c] Epidemiological Observatory, General Inspectorate of Military Health, Rome, Italy
[d] General Inspectorate of Military Health, Rome, Italy
[e] Service of Statistics, Istituto Superiore di Sanità, Rome, Italy
[f] Directorate of Digitalisation, Health Information System and Statistics, Ministry of Health, Rome, Italy
[g] Department of Health Sciences, University of Genoa, Italy
[h] Department of Experimental Medicine, University of Genoa, Genoa, Italy
[i] IRCCS Ospedale Policlinico San Martino, Genoa, Italy

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ABSTRACT

Introduction: The health profile of military veterans deployed in foreign operative theatres was assessed by several international studies because of potential exposure to depleted uranium and other pollutants. Here we reported results of 15-year epidemiological surveillance assessing long-term health effects in a cohort of Italian soldiers deployed in Iraq in 2004–2005 and participating in a biomonitoring campaign to identify potential genotoxic exposure to environmental xenobiotics before and after deployment (n = 981, SIGNUM cohort).

Methods: We evaluated mortality and hospitalization risks of the SIGNUM cohort retrospectively until 2016 and 2018 respectively. A wide cohort of military personnel never deployed abroad (n = 114,260) and the general Italian population were used as control populations in risk assessment. Causes of death and diagnoses of hospitalization were derived through deterministic record linkage with official national databases of mortality and hospital discharge. Standardized Mortality Ratio (SMR) and Standardized Hospitalization Ratio (SHR) were computed adjusting according to sex, age, area of birth, and calendar year. Differential pre-post deployment in xenobiotics concentrations and early effect biomarkers (oxidative DNA alterations and micronuclei) measured in blood serum were analysed in relation to cancer hospitalization.

Results: Mortality risk due to pathologies was more than halved compared to the general population (SMR = 0.41, 95% CI 0.11–1.05) and not significantly different compared to soldiers never deployed abroad (SMR = 0.69, 95% CI 0.19–1.68).

Similarly overall hospitalization risk due to pathologies was decreased with respect to the general population (SHR = 0.86, 95% CI 0.80–0.92) and comparable to the control military group (SHR = 0.99, 95% CI: 0.93–1.06). For haematological cancers a decreased hospitalization risk compared to the Italian general population was observed (SHR = 0.38, 95% CI 0–0.92). No statistically significant differences emerged in the patterns of biomarkers in association with cancer hospitalization.

Conclusion: The study confirms the so called 'healthy warrior' effect for the SIGNUM veterans and showed no correlation between cancer occurrence and biomonitoring markers measured on field.

1. Introduction

Military units deployed in operative theatres may undergo a variety of specific exposure to environmental stressors either physical or chemical (Geretto et al., 2021). Health surveillance of military personnel is performed by various methods including epidemiological studies,

* Corresponding author. Department of Experimental Medicine, University of Genoa, Genoa, Italy.
E-mail address: izzotti@unige.it (A. Izzotti).

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human biomonitoring, and environmental analyses. The SIGNUM study, an acronym for Genotoxic Impact Study in Military Units, was promoted by the Italian Minister of Defence in 2004 to evaluate the potential genotoxic exposure of Italian soldiers deployed in the United Nation operation in Iraq during 2004–2005 ("Ancient Babylon" operation).

The study protocol was authorized with Law n. 68 March 12th, 2004 and envisaged two distinct phases. The first phase consisted of a biomonitoring study assessing individual exposure to genotoxic xenoelements in a representative sample of military personnel before and after deployment. Environmental exposure and molecular biomarkers were examined in 981 subjects joining the study on a voluntary basis. No exposure to heavy metals or environmental xenobiotics was detected, with reference to heavy metals and depleted uranium (Bolognesi et al., 2016). The second phase consisted of ten-year clinical-epidemiological surveillance assessing long-term health effects in the cohort enrolled in the first phase (SIGNUM cohort).

The present study reports the results of the second phase of the study protocol. Mortality and hospitalization risks observed in the SIGNUM cohort were compared with expected risks in their peers not deployed in operative foreign theatres and in the Italian general population. Main pathology grouping was considered, and special attention was devoted to cancer, particularly to haematological malignancies, that originally raised suspicion about increased risk among Italian Balkan veterans (Mandelli et al., 2001). Unlike other European countries that can rely on data from long-established national cancer registries (Gustavsson et al., 2004; Storm et al., 2006; Bogers et al., 2013; Strand et al., 2014), in Italy follow-up studies on the health status of military personnel deployed abroad were done by analyzing disease notifications available to the Ministry of Defence (Peragallo et al., 2010, 2011a). Such studies, however, were limited by structural under-notification of cases for the personnel discharged from active service (Pirastu, 2011; Peragallo et al., 2011b). In the intent to obtain exhaustive figures for the whole military cohort under exam and in lack of national cancer registration coverage, we relied on official causes of death and on hospitalization administrative health records, both covering the entire national population during the follow-up time span. This also allowed to investigate a wider set of pathologies further to cancer. The methodology applied in this study can be adopted in similar circumstances where the whole population is not covered by cancer registration or not for a sufficiently long time, as is the case in several countries in Europe and worldwide.

This 15 years perspective follow-up appears unique because it is related to the analysis of molecular biomarkers of genotoxicity performed on-field during mission in the first phase of study. Accordingly, the putative association (or lack of) of these biomarkers, including DNA adducts and micronuclei, with diseases incidence and mortality was evaluated.

Obtained results were useful to address public health and policy concerns dealing with health prevention of military personnel and the use of molecular biomarkers as a predictive tool in preventive medicine.

2. Material and methods

2.1. Study design

The study was designed as a retrospective cohort study involving 981 subjects deployed between 2004 and 2005 in Iraq enrolled on voluntary basis. Urine and blood serum samples were collected before and immediately after deployment to derive individual pre-post differences in xenobiotics concentrations and early effect of biomarkers (Bolognesi et al., 2016). Informed consent was obtained from all participants. The information sheet described the aims, procedures to participate, and confidentiality measures to protect personal data. It was emphasized that participation was voluntary.

The primary objective of the study was to evaluate the long-term mortality and hospitalization (due to cancer and other pathologies of clinical relevance) observed in the SIGNUM cohort over 10 years after returning from Iraq. Information on health outcomes was derived through nominative record linkage with Italian national archives of causes of death and hospital discharges. Overall causes of death and causes related to pathologies (excluding accidents) were both considered. Only hospitalizations for acute events due to pathologies (excluding external causes), either ordinary or day-hospital admissions, were considered. Admissions for rehabilitation, long-term hospitalization, accidental causes and poisoning, pregnancy, and childbirth were therefore excluded from all analyses on hospitalizations as not related to the study purposes.

The secondary objective was to investigate the association of genotoxic exposure biomarkers measured in the SIGNUM cohort on-field at the time of deployment with long-term health conditions.

Comparisons were made against a control cohort of military personnel serving during the same time period but never deployed abroad, thus never experiencing operating conditions similar to the SIGNUM cohort. Control comparisons were also made against the Italian general population. In both cases, comparisons were adjusted by age, calendar year, sex, and birth area.

The study was approved by the ethical committee of the Italian National Institute of Health (Protocol 907/17).

2.2. Study populations

The SIGNUM cohort consisted of 981 soldiers deployed between 2004 and 2005 in Iraq. The control cohort included all military personnel belonging to the Carabinieri Army, an army corps with tasks of military police, who were in service on January 1st, 1999 (114,260 subjects) and were never deployed abroad. Carabinieri soldiers are enrolled with the same selection criteria as soldiers of the other army corps.

Controls who died or were deployed in missions abroad before 2004 were excluded. Missions abroad after 2004 for controls implied censoring of follow-up time (subjects were no more eligible as controls after an operative mission).

2.3. Data sources

Epidemiological Observatory (EOD) at the General Inspectorate of Military Health (IGESAN) of the Ministry of Defence provided:

1. Individual demographic data for the SIGNUM and control cohorts including name, gender, date of birth, municipality of birth, individual fiscal code, and the municipality of residence
2. Data on missions abroad for the control cohort, including date of departure/return, and place of deployment
3. Data collected for the SIGNUM cohort in phase-1 study regarding anamnestic information, deployment conditions (areas, tasks, and dates of deployments), genetic polymorphisms (GSTM1, XRCC1, OGG1), and results of biomonitoring exams in urine and blood serum (concentrations of Arsenic (As), Cadmium (Cd), Molybdenum (Mo), Nichel (Ni), Lead (Pb), Uranium (U), Vanadium (V), Tungsten (W), and Zirconium (Zr)) and in blood lymphocytes (DNA-adducts, 8-hydroxy-2-deoxyguanine and micronuclei frequency)
4. Death and morbidity records for the SIGNUM cohort. Follow-up data are available to EOD only for the military personnel in active service.

The Italian National Institute of Statistics (ISTAT) is the source for:

1. Official causes of mortality from 2004 to 2016 including all deaths that occurred in Italy. Causes of death are coded according to the tenth edition of the International Classification of Disease (ICD-10) with a standardized methodology all over the country and over time.
2. Resident population by calendar year, age, sex, residence, and birth area

The Italian Ministry of Health, in the framework of collaboration agreements with the Ministry of Defence and the Italian National Institute of Health, provided:

1. Hospital Discharge Records (HDR) from the national database including all hospital admissions 2005–2018 of SIGNUM and control cohorts. The hospitalization record included main diagnosis and up to five secondary diagnoses. All diagnoses were coded according to the ninth revision of the International Classification of Diseases – Clinical Modification (ICD-9CM). Only the main diagnosis of hospitalization was considered in the study.
2. Hospitalization risks for the Italian general population by demographic characteristics in the period 2005–2017

2.3.1. Record linkage

Deterministic record linkage with the official mortality database was carried out to ascertain vital status for all subjects in the study and control cohorts during 2004–2016. Linkage keys were fiscal code, name, surname, sex, place, and date of birth. For all detected deaths ICD-10 causes of death coded by ISTAT were retained.

Deterministic record linkage with the national archive of HDR in the period 2005–2018 was performed by the Ministry of Health for both cohorts. A unique linkage key based on the fiscal code was used.

Results of the record linkage process were compared with mortality and morbidity information available in the EOD database (bullet point 4). A cross-check was carried out between diagnoses of hospitalization and causes of death.

2.4. Statistical analysis

Standardized Mortality Ratio (SMR) and Standardized Hospitalization Ratio (SHR) were used to compare the observed risk of mortality or hospitalization in the SIGNUM cohort with the corresponding expected risk derived from control populations (Carabinieri cohort or general population). SMR and SHR are the ratio of observed to expected number of events (deaths or hospitalization) in the target cohort over the whole follow up time. In these indirect standardizations the expected risk was stratified by age (10-years age groups: 20–29 to 70–79), calendar year, sex, and area of birth. Two macro-areas following ISTAT classification were used: North/Centre and South/Islands/Abroad. The foreign area of birth was assimilated to Southern Italy in consideration of past migration flows. In each of these strata the expected number of events is derived multiplying person-time at risk in the SIGNUM cohort by the observed event rate in the control population (observed events divided by person-time at risk).

Wald 95% Confidence Intervals (CIs) for SMR/SHR estimates were calculated by assuming observed deaths/hospitalizations followed a Poisson distribution. Risk comparison between the SIGNUM cohort and control populations was based on SMR/SHR estimates and their corresponding 95%CIs. If the confidence interval does not include the unit value, the difference between the groups was considered statistically significant. In the analysis of biomonitoring data, non-parametric tests were used for comparisons between groups (Chi-Squared and Fisher Exact test in case of categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test in case of continuous variables), all tests were 2-sided, accepting $p < 0.05$ as indicating a statistically significant difference. Analyses were done with SAS version 9.4 and STATA version 13 statistical packages.

2.5. Standardized Mortality Ratio (SMR)

Person-time at risk was accumulated from the starting date of the mission in Iraq (SIGNUM cohort) or from 01/01/2004 (Carabinieri cohort) to death or end of follow-up (31/12/2016). Carabinieri soldiers deployed in equivalent Iraq theatres during the follow-up were censored at the mission starting date.

SMR values based on the control cohort could be sensitive to low person-time at risk in some standardization strata. To assess this effect a sensitivity analysis was carried out by comparing SMR derived from model-based (Poisson-distributed) and observed expected death risks in the control cohort.

2.6. Standardized Hospitalization Ratio (SHR)

Person-time at risk was accumulated from 01/01/2005 (starting time of HDR electronic archive in Italy) to death or end of follow-up (31/12/2018). Two distinct indicators were considered with distinct censoring criteria:

- Overall hospitalization: total number of admissions for all eligible causes. Multiple events for each person were included and the first hospitalization was not a reason for censoring the follow-up time.
- Cause-specific hospitalization: number of people hospitalized for a given cause (including only the first cause-specific admission in case of multiple events for the same cause for each person). The first hospitalization for the cause in the exam was a censoring event because only one admission was retained. A subject with admissions for multiple causes (differences up to the third digit of ICD9-CM classification were considered) contributed to the calculation of each cause-specific hospitalization rate with distinct follow-up times.

A mission abroad in operative theatres was an additional censoring cause for the Carabinieri cohort.

When comparing against the Carabinieri cohort, in the case of rare diseases (less than 50 observed first hospitalizations during the whole follow-up time) SHR was not adjusted by calendar year, as the limited size of the cohort impairs robust estimation of annual hospitalization risk. Conversely, comparisons against the Italian male population were standardized with the same detail for all pathologies (no sample size limits).

Table 1
Characteristics of the SIGNUM cohort and of the military control cohort of Carabinieri. Age is referred to the date of first mission in Iraq (SIGNUM cohort) and to 1/1/1999 (Carabinieri control cohort).

Number of subjects		SIGNUM	Carabinieri
		981	114,260
Age - mean (SD)		28.18 (6.12)	37.10 (8.1)
Age classes (%)	20–29	671 (68.4)	20,792 (18.3)
	30–39	233 (23.8)	46,832 (41.1)
	40–49	69 (7.0)	38,575 (33.9)
	50–59	8 (0.8)	6,386 (5.6)
	60–69	0 (0.0)	1,304 (1.1)
	70–79	0 (0.0)	2 (0.0)
	80–89	0 (0.0)	7 (0.0)
Sex (%)	Female	29 (3.0)	2 (0.0)
	Male	952 (97.0)	114,258 (100.0)
Army Corps (%)	Air	14 (1.4)	0 (0)
	Carabinieri	187 (19.1)	114,260 (100)
	Army	630 (64.2)	0 (0)
	Navy	150(15.3)	0 (0)
Area of birth (%)	Italy, Centre (C)	119 (12.1)	20,199 (17.7)
	Abroad (A)	31 (3.2)	2,924 (2.6)
	Italy, Islands (I)	164 (16.7)	23,384 (20.5)
	Italy, North (N)	91 (9.3)	21,164 (18.5)
	Italy, South (S)	576 (58.7)	46,589 (40.8)
Area of birth, groups (%)	N/C	210 (21.4)	41,363 (36.2)
	S/I/A	771 (78.6)	72,897 (63.8)

3. Results

3.1. Characteristics of the study population

The SIGNUM cohort consists of 981 subjects, men in the great majority (97.0%) (Table 1). Approximately 68% of them were aged 20–29 at the time of their first mission in the area, about 24% were aged 30–39 and about 8% were over 40. The mean age of enrolment in the cohort was 28.2 years (SD = 6.1). They mostly belong to Army Corps (64%) and 19% of them were Carabinieri.

The control cohort of Carabinieri included 114,260 subjects, all men except two women. In 2004 most subjects were aged between 30 and 39 years (41.1%) with a mean age of 37.1 years (SD = 8.1).

Most subjects were born in Southern Italy: 58.7% in SIGNUM and 40.7% in the Carabinieri cohort respectively. A small, but not negligible proportion (about 3%) was born overseas.

3.2. Data selections and linkage validation

Out of the 114,260 soldiers in the Carabinieri control cohort, the following subjects were excluded: 9 subjects over 70 years old, 362 subjects died before 2004, 5,685 subjects with missions abroad prior to 2004, and 173 subjects also belonging to the SIGNUM cohort (both two last groups included invalid controls). During the follow-up, further 6,125 subjects were censored due to missions abroad. Additional 430 subjects with missing or incomplete fiscal code had to be excluded from the hospitalization study only, as not linkable with HDR data.

As for the SIGNUM cohort, subjects belonging to the Carabinieri corps (187 out of 981) were excluded from the analyses regarding hospitalizations for thyroid cancer because an ultrasound screening program addressed specifically to personnel deployed abroad was established in 2005 by the Carabinieri corps.

Through the comparison with the EOD database, 2 deaths in the SIGNUM cohort occurring abroad and not recorded in the Official Mortality archive were identified. Seven of the eight overall deaths resulting from the linkage were found also in the EOD database. All deaths due to cancer had been hospitalized for cancer.

Out of the 23 tumors resulting from the hospitalization study, 13 were in common with EOD data while 10, partly related to soldiers no more in service, were only identified through HDR data.

3.3. Mortality results

Overall, in the period 2004–2014, 10 deaths were identified in the SIGNUM cohort, two of them occurred abroad (source EOD) and 8 in Italy (source ISTAT). Deaths from causes related to trauma and accidents were the majority (6 out of 10), as expected in the young male population. The remaining 4 deaths due to pathology were caused by: 2 malignant tumors (stomach and lung cancers), myocardial infarction, and aplastic anaemia.

Observed mortality due to pathology in the SIGNUM cohort was lower than in soldiers never deployed abroad, although the difference was not statistically significant (5.7 expected deaths, SMR = 0.69, 95% CI 0.19–1.68). On the other hand a substantially decreased risk emerged in comparison to the general population (9.8 expected deaths, SMR = 0.41, 95% CI 0.11–1.05) (Table 2).

Observed overall mortality in the SIGNUM cohort did not significantly differ from that in the Carabinieri cohort (8.8 expected, SMR = 1.13, 95% CI 0.43–1.83) and resulted 28% decreased as compared to the general population (14 expected, SMR = 0.72, 95% CI 0.27–1.17) (Table 2).

The sensitivity analysis on the SMR computation method did not show any significant difference compared to the standard analysis.

Notably, mortality risk due to pathology in the Carabinieri control cohort was significantly lower than that expected in the Italian general population of comparable demographic characteristics (1,539 observed deaths compared to 2,503 expected) with SMR = 0.61 (95% CI 0.58–0.65).

4. Hospitalization results

4.1. Overall hospitalizations

In the period 2005–2018, the total number of hospitalizations detected in the SIGNUM cohort was 799 (Table 3). Hospitalization risk was equivalent to that expected in a comparable group from the control cohort of Carabinieri (803 expected, SHR = 0.99, 95% CI 0.93–1.06).

Overall hospitalization risk in the SIGNUM cohort was instead significantly lower (SHR = 0.86, 95% CI 0.80–0.92) than the risk expected in the general Italian population paired on the same demographic characteristics.

4.2. Major diagnoses of hospitalization

In the period 2005–2018 the first five diagnoses of hospitalization in the SIGNUM cohort were related to diseases of the musculoskeletal system (127 subjects with at least one hospital admission), digestive system (82), circulatory system (79), and genito-urinary system (58) (Table 3).

Observed hospitalization risk for musculoskeletal system diseases was slightly higher than the expected risk in both the Italian population (SHR = 1.34, 95% CI 1.10–1.57) and the Carabinieri cohort (SHR = 1.27, 95% CI 1.06–1.51). As for digestive system diseases, hospitalization risk was significantly lower than expected, both using the Carabinieri cohort (SHR = 0.78, 95% CI 0.62–0.97) and the Italian general population (SHR = 0.61, 95% CI 0.47–0.74).

Comparable risk was found in cardiovascular system pathologies against both reference populations.

For the genitourinary system diseases, the risk was significantly increased compared to the military control population, and slightly lower than in the general population.

4.3. Hospitalizations due to malignant tumors

In the period 2005–2018 the admissions with main diagnosis of malignant tumor in the SIGNUM cohort were in total 23, corresponding to 22 first hospitalizations for cancer in 22 distinct subjects (one soldier had two admissions for two different primary cancers). In order of

Table 2
Observed and expected deaths 2004–2016 in the SIGNUM cohort by cause of death and reference population. Standardized Mortality Ratio (SMR) with 95% confidence interval (95% CI).

Cause of death	Reference Population	Number of deaths		SMR	95% CI	
		observed	expected		Lower	Upper
Overall mortality	Carabinieri	10	8.8	1.13	0.43	1.83
Mortality due to pathologies[a]	Carabinieri	4	5.7	0.69	0.19	1.78
Overall mortality	Italian population	10	13.9	0.72	0.27	1.17
Mortality due to pathologies[a]	Italian population	4	9.8	0.41	0.11	1.05

[a] All causes of death except accidents.

Table 3
Number of observed and expected hospitalizations in the SIGNUM cohort by main diagnosis and reference population. Standardized Hospitalization Ratio (SHR) with 95% confidence interval (CI).

Diagnosis (ICD9-CM codes)	Carabinieri Control cohort (2005–2018)					Italian General Population (2005–2017)				
	Hospitalizations		SHR	95% CI		Hospitalizations		SHR	95% CI	
	observed	expected		lower	upper	observed	expected		lower	upper
Overall hospitalizations[b]	**799**	**802.8**	**0.99**	**0.93**	**1.06**	**750**	**873**	**0.86**[a]	**0.80**	**0.92**
Major hospitalizations diagnoses										
Circulatory system (390–459)	79	75.1	1.05	0.83	1.31	78	86.7	0.90	0.70	1.10
Digestive system (530–579)	82	105.1	0.78[a]	0.62	0.97	79	130.4	0.61[a]	0.47	0.74
Genitourinary system (580–629)	58	41.4	1.40[a]	1.06	1.81	52	57.1	0.91	0.66	1.16
Muscoloskeletal system (710–739)	127	99.8	1.27[a]	1.06	1.51	122	91.3	1.34[a]	1.10	1.57
All malignant cancers (140–172, 174–208)	22	16.2	1.36	0.85	2.06	20	23.1	0.86	0.53	1.34
Main cancer diagnoses										
Testis cancers (186)	4	2.4	1.68	0.46	4.31	4	2.6	1.56	0.03	3.10
Brain cancers (191)	3	0.8	3.90	0.80	11.40	2	1.1	1.81	0	4.32
Thyroid cancers (193)	3	1.5	2.05	0.42	5.99	3	1.5	1.36	0.16	4.91
Haematological tumors (200–208)	2	2.2	0.90	0.11	3.25	2	5.2	0.38[a]	0	0.92
Hodgkin's Lymphoma (201)	0	0.4	0	0	8.26	0	1.3	0	0	2.84
Non Hodgkin Lymphomas (200, 202)	1	0.8	1.18	0.03	6.59	1	2.2	0.45	0	1.33
Leukaemias (204–208)	1	0.7	1.52	0.04	8.49	1	0.4	2.74	0	8.10

[a] Statistically significant variation at 95% confidence level.
[b] Total number of hospitalizations for acute events due to pathologies (excluding external causes). Multiple hospitalizations for a given cause for the same person are included.

frequency, hospitalizations for malignant cancers regarded: testis (n = 4), brain (n = 3), thyroid (n = 3), colon (n = 2), urinary bladder (n = 2), haematological tumors (n = 2), stomach, lung, gallbladder and intrahepatic bile ducts, prostate, skin melanoma, tumor with an unknown primary site (n = 1).

Hospitalization risk for all cancers combined in the SIGNUM cohort (Table 3), although not significantly, was slightly higher than in the military control cohort (22 vs 16.2 expected, SHR = 1.36, 95% CI 0.85–2.06) and slightly reduced compared to the Italian general population (20 observed hospitalizations in the period 2005–2017 against 23.1 expected, SHR = 0.86, 95% CI 0.53–1.34).

Cancers with early age at onset, i.e., testis, brain, and thyroid, were the most frequently diagnosed (10 overall). For all these three cancers the estimated excess hospitalization risk was subject to high statistical uncertainty due to the small number of observed events.

Focusing on all haematological tumors, the hospitalization risk was significantly lower compared to the Italian general population (SHR = 0.38, 95% CI 0–0.92) and comparable to that in the Carabinieri cohort, although with high statistical uncertainty (SHR = 0.90, 95% CI 0.11–3.25).

There was no evidence of significantly increased risk for non-Hodgkin's lymphoma, leukemia, and Hodgkin's lymphoma (no hospitalizations observed in the SIGNUM cohort) against both control populations. (Table 3).

4.4. Analysis of biomonitoring data

Differential pre-post deployment in biomarkers of exposure (xenobiotics concentrations) and early effect (oxidative DNA alterations and micronuclei) measured in blood serum were compared between subjects hospitalized for malignant cancers (N = 20) or not (N = 830). Complete pre-post-measures were indeed available for 850 out of 981 total subjects. This comparison did not show statistically significant differences between the two groups (Table 4), except for Molybdenum for which median pre-post differential in blood serum concentration was significantly lower in those hospitalized for cancer.

5. Discussion

5.1. Mortality results

Compared to their peers never deployed abroad, the risk in the SIGNUM cohort, although not significantly, resulted slightly increased for the overall mortality and decreased for the mortality due to pathologies. This result is consistent with the fact that most observed deaths in the SIGNUM cohort occurred for accidental causes (6 deaths out of 10) both in operative theatre and in car accidents.

Compared to the general population, mortality in the SIGNUM cohort was 28% and 59% lower when considering respectively all causes of death or all causes except accidents. Although these differences didn't fully reach statistical significance such lower mortality rates are in line with the established 'healthy soldier effect', a term indicating the matter of fact that soldiers deployed in operative theatres are accurately selected for their good health and physical performances. An overall meta-analysis of 59 studies reported an average healthy soldier effect accounting for 10–25% as compared to the general population (McLaughlin et al., 2008). The healthy soldier effect observed in the SIGNUM cohort was slightly higher (28%) but consistent with results from the literature (especially if considering the limited cohort size). Notably, the Carabinieri cohort resulted to have a lower mortality risk compared the Italian general population, thus confirming it an appropriate reference group for the SIGNUM cohort.

5.2. Hospitalization results

The number of overall hospitalizations for acute events due to pathologies was significantly lower by 14% in the SIGNUM cohort as compared to the age-standardized Italian population, in accordance with mortality results. No difference was observed as compared to the military reference cohort. When considering hospitalizations for main nosological groupings, some differences emerged. Injuries of the musculoskeletal system were the most frequent hospitalization cause and showed a weakly significant excess risk compared to both control populations. This finding was in line with other studies reporting the injuries of the musculoskeletal system, with particular reference to those affecting meniscus and cruciate ligaments of the knee, as most frequent hospitalization cause in active-duty military service members (Jones et al., 2012). Such lesions increase with age and are more frequent in

Table 4
Median (25%, 75%) differentials in biomarkers measured pre-post deployment in blood serum. Overall values and comparison between subjects hospitalized or not for malignant cancer.

Biomarker	Overall	Not hospitalized for malignant cancer	Hospitalized for Malignant cancer	
	N = 850	N = 830	N = 20	p-value
Xenobiotic in blood serum (microgr/l)				
As	−0.10 (−0.80, 0.30)	−0.10 (−0.90, 0.30)	0.00 (−0.52, 0.22)	0.61
Mo	0.00 (−0.20, 0.30)	0.00 (−0.20, 0.30)	−0.10 (−0.40, 0.02)	**0.021**
U	−0.008 (−0.022, 0.005)	−0.008 (−0.022, 0.005)	−0.007 (−0.022, 0.009)	0.86
V	0.00 (−0.03, 0.01)	0.00 (−0.03, 0.01)	0.00 (−0.02, 0.02)	0.47
W	−0.01 (−0.06, 0.01)	−0.01 (−0.06, 0.01)	−0.01 (−0.06, 0.01)	0.92
Zr	0.02 (−0.12, 0.15)	0.02 (−0.12, 0.15)	−0.04 (−0.08, 0.11)	0.42
Oxidative Dna alterations				
Dna Adducts	−0.06 (−0.39, 0.29)	−0.05 (−0.38, 0.29)	−0.12 (−0.42, 0.26)	0.73
8-Oxo-dg	0.10 (−0.19, 0.89)	0.10 (−0.19, 0.91)	−0.01 (−0.22, 0.48)	0.3
Micronuclei				
BN%	7 (−1, 15)	7 (−1, 15)	8 (3, 16)	0.49
CBP	0.17 (−0.01, 0.33)	0.17 (−0.01, 0.33)	0.20 (−0.09, 0.28)	0.62
BNMN_BN	0.5 (−1.2, 2.5)	0.5 (−1.3, 2.5)	1.5 (−1.0, 3.0)	0.43
MN_BN	0.5 (−1.4, 3.0)	0.5 (−1.4, 2.9)	1.7 (−1.0, 3.5)	0.36
MONO	0.00 (−0.50, 1.00)	0.00 (−0.50, 1.00)	0.07 (−0.50, 1.32)	0.47
MN_MONO	0.00 (−0.99, 1.12)	0.00 (−1.00, 1.12)	0.23 (−0.50, 1.44)	0.51
BUD_BN	0.00 (−0.50, 0.00)	0.00 (−0.50, 0.00)	0.00 (0.00, 0.00)	0.63
NPB	0.00 (−1.00, 0.48)	0.00 (−1.00, 0.48)	−0.28 (−0.75, 0.00)	0.75
MN&NPB_BN	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.37
NPB_BN	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.57

BN%: percent proportion of binucleated cells.
CBP: Cytokinesis Block Proliferation Index.
BNMN_BN: number of binucleated cells (BN) with micronuclei (MN) out of 1000 BN cells analysed.
MN_BN: number of micronuclei out of 1000 binucleated cells analysed.
MONO: number of mononucleated cells (MONO) with micronuclei out of 1000 MONO cells analysed.
MN_MONO: number of total micronuclei out of 1000 mononucleated cells analysed.
BUD_BN: number of binucleated (BN) cells with bud out of 1000 BN cells analysed.
NPB: number of nucleoplasmic bridges (NPB) out 1000 binucleated cells analysed.
MN&NPB_BN: number of binucleated (BN) cells with micronucleus and nucleoplasmic bridge (NPB) out of 1000 BN cells analysed.
NPB_BN: number of binucleated cells with nucleoplasmic bridge (NPB) out of 1000 total binucleated cells.

males than in females. This situation was amenable to the peculiarities of physical activities occurring during operative military service either during hard training or missions in operative theatres.

The risk of hospitalization due to digestive system diseases was significantly lower both as compared to the Italian population as well as the military reference cohort. This result was unexpected since personnel deployed in operative theatres could be exposed to a high risk of orofecal transmitted infection. This finding suggests that the logistic setup of health and preventive measures to control these diseases, including water potabilization and availability as well as food processing and storage, was effective.

5.3. Hospitalizations due to malignant tumors

Results provide uncertain evidence of differences in the hospitalization risk for cancer. The most frequent tumors were those at early-onset (testis, brain, and thyroid cancers), consistently with the young median age of the cohort. The small cohort size and the rarity of these tumors (incidence rate below 6 per 100,000) imply a large statistical uncertainty of the estimated excess risk compared to both controls. As for tumors of the hematopoietic system, no difference was found as compared to the military reference cohort, while a significantly lower incidence rate (62%) was observed against the Italian population. No increased risk in the hospitalization frequency of Lymphoma, either Hodgkin or non-Hodgkin, was detected in the SIGNUM cohort as compared to both the Carabinieri cohort and the Italian population. These results did not support the existence in the examined cohort of any relationship between the military service in operative theatre and arising of onco-hematological malignancies in the following years. This finding was in line with other similar studies showing no evidence of increased haematological malignancies after deployment. The cancer incidence in 18,175 Dutch Balkan veterans after 15 years follow-up was 17% and 15% lower than those observed in 135,355 undeployed military peers and in the Dutch general population, respectively (Bogers et al., 2013).

This finding was also consistent with a similar follow-up study analyzing mortality of soldiers deployed in the Balkan theatre against Carabinieri serving in Italy and the general population (Capocaccia et al., 2016). This study showed no evidence of increased death risk for all causes and for malignant cancers in the veterans compared to both control populations. Notably, the study relied on individual linkage with the national causes of death archive and followed a methodology fully comparable with the SIGNUM cohort epidemiological assessment. Conversely complete and standardized coverage of morbid events occurring in the target population could not be granted in studies based on disease (self-) notifications in the military personnel (Mandelli et al., 2001; Peragallo et al., 2011a). This represented a major methodological difference limiting the comparability of such studies with the results obtained in our approach.

The overwhelming evidence of increasing overdiagnosis of thyroid cancers, mainly small papillary carcinomas, with increasing active surveillance of the population (Vaccarella et al., 2015, 2016) motivated the non-eligibility in thyroid cancer analyses of the SIGNUM veterans enrolled in the Carabinieri army (N = 187). A specific ultrasound screening protocol after deployment was foreseen for Carabinieri veterans whereas no similar procedures were in place for veterans of the other army. Screening for thyroid cancer in asymptomatic adults was not recommended in most countries because of the lack of clear evidence of the benefits versus the harms of overdiagnosis and overtreatment (Bibbins-Domingo et al., 2017).

5.4. Analysis of biomonitoring data

A further goal of our study was to evaluate the long-term outcome in the SIGNUM cohort in relation to the biomonitoring data measured before and after the mission in order to evaluate potential associations. To the best of our knowledge, the SIGNUM project was the widest study in this regard so far performed. Indeed, molecular biomarker analysis was performed in veterans as related to mission in operative theatre (Iraq) in 35 US soldiers directly exposed to depleted uranium because of friend fire (McDiarmid et al., 2015), 199 UK soldiers (Bland et al., 2007), and 103 Canadian soldiers (Ough et al., 2002). In these studies, only

environmental exposures biomarkers (urinary isotopes) were analysed. Conversely, the SIGNUM study enrolled 981 Italian soldiers analysed both before and immediately after the mission in the operative theatre. Further to exposure biomarkers, a wide variety of early response (e.g., DNA adducts, oxidative stress) and early damage (e.g., micronuclei), and susceptibility (gene polymorphisms) biomarkers were analysed on the whole accounting for more than 8,000 analyses. It is noteworthy that this effort was performed although no direct exposure of Italian military forces to depleted uranium was recorded, unlike for US forces. Furthermore, the SIGNUM study was the only one evaluating the relationship between biomarkers of exposure and disease incidence resulting from the epidemiological follow-up.

We found no significant correlation between median variations in biomarkers analysed pre-post deployment in 2004–2005 and cancer hospitalization rate up to 2018. This result indicated that the observed biomarkers modulation was neither predictive nor related to cancer occurrence, since subjects who did and did not develop cancers experienced similar average differentials in biomarkers. Interestingly, an inverse association, although not significant, was detected for oxidative stress biomarker, the only one altered in the whole cohort during operative deployment. This finding provided evidence that oxidative stress was an adaptive event not representing, at the low level detected, a risk factor for disease occurrence but only indicating a stress situation properly faced by the defensive mechanism of the organism (Rossnerova et al., 2020).

The only statistically significant relationship regarded a slight median reduction in molybdenum concentration for cancer hospitalized subjects of the SIGNUM cohort. It should be noted that molybdenum median pre-post differential concentration was very low in both groups, being around zero for the whole cohort. Molybdenum was neither genotoxic nor mutagenic and was not able to induce micronuclei formation in human cells (Burzlaff et al., 2017). A recent extensive study demonstrated that there was no association between plasma levels of molybdenum and risk for cancer development (Bai et al., 2019). IARC has classified cadmium and Nickel compounds as carcinogenic to humans (Group 1). Vanadium pentoxide as a Group 2B (possible) human carcinogen and Tungsten and Lead are probably carcinogenic in humans (Group 2A).

6. Conclusions

In conclusion, the epidemiological long-term assessment of health profile of subjects enrolled in the SIGNUM cohort confirmed the so-called 'healthy warrior' effect and did not support the evidence of an increased risk with respect to the military cohort never deployed abroad in terms of mortality and overall hospitalization due to pathologies.

Furthermore, hospitalization rate related to cancers resulted to be not significantly different from the expected rates in both control populations, except for haematological malignancies whose hospitalization risk was lower than that expected in the Italian general population.

The small size of the cohort, even if followed up for more than 10 years, limits the analysis of rare disease conditions making estimates more prone to random variability. Volunteer bias may have implied some distortions of the estimated health profile, however no specific data were available to assess in which direction. Risk adjustment by life style factors wasn't possible as well.

The study design peculiarities corroborating the scientific soundness of these findings were: the individual linkage with official national archives granting complete (and equal) coverage of target and control cohorts, the use of standardized information uniformly coded all over the country on the causes of death and hospitalization, and the double control population.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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