Trends in testicular germ cell cancer incidence in Eastern Croatia

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ABSTRACT

Aim To investigate a possible association between radioactive and toxic elements contamination, mainly depleted uranium (DU) from the Chernobyl accident, Croatian War of Independence and Bosnian War and the increasing incidence of testicular germ cell cancers (TGCC) in the population of Eastern Croatia.

Methods From 1969 to 2012, 258 testicular cancer (TC) patients were treated at the Department of Urology, University Hospital Centre Osijek. Incomplete data were found in 32 patients who were excluded from the analysis and 10 patients had non-TGCC TC. Seminoma and non-seminoma groups were included out of 216 TGCC patients. The patients were assigned to one of the time periods: 1969-1995 (distant prewar and war period) and 1996-2012 (postwar period).

Results In the postwar period 3.5 times higher incidence rate for non-seminomas (4.5 patients yearly vs. 1.3), seminomas (4.2 vs. 1.2) and TGCC overall (8.7 vs. 2.5) was found compared to the prewar period, with non-seminoma presenting in more advanced stage III (35.5% vs. 13.9%, p=0.013).

Conclusion Usage of depleted uranium in armed conflicts could lead to the development of TGCC after unknown time of latency. Exposure assessment is mandatory to determine a possible causative correlation between the depleted uranium exposure and testicular germ cell cancer.

Key words: testicular neoplasms, nuclear warfare, uranium, Chernobyl nuclear accident, Croatia
INTRODUCTION

Testicular cancers (TC) are in 90-95% testicular germ cell cancers (TGCC) (1), which can histologically be divided into seminomas and non-seminomas, both having their distinctive clinical course and prognosis. The incidence of TGCC is increasing worldwide and varies considerably in different geographical regions, between different ethnic groups, even within a relatively homogeneous population such as Europe. The incidence is among the highest in some Scandinavian countries, Switzerland, Germany and New Zealand, intermediate in the United States and Great Britain and lowest in Africa and Asia (2-5). Denmark has the highest incidence rate (9.9/100 000) in contrast to Sweden (5.0/100 000) and Finland (2.7/100 000). Asian and black populations have much lower incidence rates, while the rate in New Zealand is relatively high (7.1/100 000) (4). The etiology of TGCC is still unknown and it is difficult to explain why the incidence rates vary so much. Studies have shown that during embryogenesis of affected individuals TGCC develops as a precursor lesion that is affected by many prenatal and postnatal factors that are essential for the progression of the disease (6). Generally accepted etiological factors that can lead to the development of TGCC are cryptorchidism, age, contralateral TGCC, gonadal dysgenesis and genetic abnormalities (7). It is unlikely that the errors in the classification of other tumors are responsible for changes in the rate of incidence for TGCC because they are histologically well-characterized (8). Studies report that recent increases in the incidence of TC could be attributed to birth-cohort effects, which implies that diet, endocrine disrupters and other environmental factors may play a major role in TC carcinogenesis (9-11).

Patients with TC from Eastern Croatia gravitate to the Department of Urology in Osijek and there is an increase in TGCC incidence, with stage-shifting towards advanced stages in the last 40 years. Events which severely affected the vast majority of our population, meaning Chernobyl accident in 1986, Croatian War of Independence (1991-1995) and Bosnian War (1992-1995) could be the possible explanation for the observed trend. Chernobyl’s radioactive cloud contaminated the soil in every country in the Northern Hemisphere (12). Scientific and epidemiological research on the effects of the Chernobyl accident (13,14) and Gulf War (15,16) has shown a rising incidence of various malignant diseases, including TGCC. Despite the rising TGCC incidence among non-military personnel in North European countries (17-19) an increase was also observed in the Gulf War (15) and the Balkan War veterans involved in Kosovo War (1998-1999) (20,21). Radioactive and toxic elements from ammunition, with emphasis on depleted uranium (DU) were pointed out as possible causative agents in the Gulf and Kosovo War (22,23).

Most common usage of DU is in coating bullets and shells to improve armor-piercing capabilities (24). Medical effects of internal exposure to DU include nephrotoxicity, reproductive and developmental toxicity, as well as mutagenic and carcinogenic effects (25,26). The NATO officially acknowledged the use of DU ammunition in the Bosnian and Kosovo War. Data show that 10 000 30 mm DU rounds (3.3 tons of DU) were fired at 12 sites in Bosnia and 31 000 DU rounds (10.2 tons of DU) at 85 locations in Kosovo (27,28). Unfortunately, there are no data on the amount of DU rounds used in the Croatian War of Independence. Recent studies show that at impact ammunition particles disperse into the air and contaminate the soil, ground water and river systems (29,30). Internal exposure occurs by ingestion of contaminated food and water or by inhalation of particles resuspended in the atmosphere (31,32). Environmental contamination with toxic and radioactive elements, together with war-related stress has the potential of being one of the important causative agents in the genesis of TGCC. Therefore, the aim of this study was to point out the possible connection between the abovementioned and the change in TGCC incidence and stage presentation in our population.

PATIENTS AND METHODS

From 1969 to 2012 258 TC patients were treated at the Department of Urology, University Hospital Centre Osijek, Croatia. Data were retrospectively collected and analyzed from patient’s medical history, operating protocols and pathohistological findings. Medical charts of 226 patients were used in the analysis, since 32 patients had incomplete or missing data. After initial radical orchietomy additional diagnostic procedures available at the time (bipedal lymphography, abdominopelvic CT, retroperitoneal lymph node dissection, chest X-ray or chest CT where indicated, serum tumor
markers) were performed to assess the proper stage of the disease. Methods of treatment included radical orchiectomy, followed by either surveillance, retroperitoneal lymph node dissection and/or oncological treatment (chemo/radiotherapy). Staging and histology were assessed according to internationally accepted criteria, with TNM(S) staging and stage grouping (1). Follow-up was performed on an outpatient basis.

Overall, 216 TGCC patients were divided into seminoma and non-seminoma group; 10 non-TGCC patients were excluded from further analysis. To compare the changes in TGCC incidence two time periods were artificially created: 1969-1995 (distant prewar and war period) and 1996-2012 (postwar period). Descriptive statistical analysis with Fisher Exact and Kolmogorov-Smirnov test for comparing different variables between the groups was performed. Trends in cancer incidence were analyzed with curve estimation regression model. A p-value of <0.05 was considered statistically significant.

**RESULTS**

Seminoma was found in 104 (46%) and non-seminoma in 112 (49.6%) patients. Only 4.4% of TC’s were of non-germ cell origin and among 10 of them 6 were Non-Hodgkin lymphomas. Only TGCC’s were included in further analysis. Non-seminomas were mixed TGCC in 65.2%, embryonal carcinoma in 25.9%, with teratomas, yolk sac tumors and choriocarcinomas occurring rarely. As expected, seminoma patients were older than non-seminoma patients. Right sided TC was more common in non-seminoma group. Seminoma and non-seminoma ratio was 0.89 for 1969-1995 period and 0.95 for 1996-2012 period (p=0.472). Demographic and clinical data with stage distribution between the periods for all TGCC, seminoma and non-seminoma are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical data of patients with testicular germ cell cancer (TGCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Median age (range)</td>
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<tr>
<td>Right side (%)</td>
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<tr>
<td>Period 1969-1995</td>
</tr>
<tr>
<td>Stage I (No, %)</td>
</tr>
<tr>
<td>Stage II (No, %)</td>
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<tr>
<td>Stage III (No, %)</td>
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<tr>
<td>Period 1996-2012</td>
</tr>
<tr>
<td>Stage I (No, %)</td>
</tr>
<tr>
<td>Stage II (No, %)</td>
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<tr>
<td>Stage III (No, %)</td>
</tr>
</tbody>
</table>

The rise in the incidence of TC in the studied population over the years was found. There were two distinctive rises and new plateau was reached in 1996 and again in 2005 (Figure 1). When analyzing the change in the incidence of TGCC between the two pre-selected periods, 3.5 times higher incidence rate was found in the postwar period for non-seminomas, seminomas and TGCC overall (Table 2). Curve fit and estimation regression analysis showed that rise in the incidence of all TC, seminomas and non-seminomas, assuming linear annual rise, was significantly different than expected (p<0.000) (Figure 2).
Furthermore, TGCC stage distribution between the periods was studied. For seminomas there were no statistically significant changes in the stage presentation of the disease, although there were more stage I and less stage II and III patients in the post-war period. Unlike seminomas, non-seminomas have shown a significant stage shift in the second period, with more patients presenting in the advanced stage III of the disease on account of less stage II patients, which reflected itself on the stage shift results for TGCC overall (Table 3).

**DISCUSSION**

The incidence of TGCC in the analyzed sample was rising through the years, with the significant rise in the 1996-2012 period, after the events which could have had an impact on TGCC development. Regarding the trend, the curve is growing faster than expected. The reason for seminoma presenting in less advanced stage while non-seminoma presents in more advanced stage in 1996-2012 period is still an enigma.

There is much controversy in the scientific community regarding the “Gulf War Syndrome” and the “Balkan War Syndrome”. After the Persian Gulf War ended there was a great concern that the U.S. and U.K. war veterans developed negative health consequences including increased mortality rate. Higher mortality rate was noticed among them in comparison to veterans who were deployed elsewhere, but this increase was caused by the death from external causes, in most cases by trauma, not from the disease-related causes (33,34).

The knowledge of the late effects of long term exposure to radiation is limited because dose-response assessments are based on studies of exposure to high doses of ionizing radiation and animal experiments. It is difficult to detect an increase in cancer incidence or mortality because the majority of exposed individuals received low doses of radiation, comparable to or few times higher than annual levels of natural background radiation (35).

Uranium \( (92\text{U}) \) is the heaviest naturally-occurring element found at low levels in soil and water, but in significant concentrations in rock deposits and uranium-rich ores. It has radioactive and fission properties and therefore it is one of the most harmful chemical elements in nature to human health (36-38). There are three different isotopes of \( _{92}\text{U} \) found in nature, 99.27% uranium-238 (U-238), 0.75% uranium-235 (U-235) and 0.0055% uranium-234 (U-234) (36). Uranium isotopes can be separated to increase the concentration of one isotope relative to another. This process is called “enrichment” (36). The process produces huge quantities of \( _{92}\text{U} \) that are depleted in U-235, but are almost pure U-238, called DU (36). In the mi-

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**Table 3. Testicular germ cell cancer (TGCC) stage shift between the periods**

<table>
<thead>
<tr>
<th></th>
<th>1969-1995</th>
<th>1996-2012</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All TGCC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>36 (52.9)</td>
<td>81 (54.7)</td>
<td>0.460</td>
</tr>
<tr>
<td>Stage II</td>
<td>24 (35.3)</td>
<td>34 (23)</td>
<td>0.043</td>
</tr>
<tr>
<td>Stage III</td>
<td>8 (11.8)</td>
<td>33 (22.3)</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Seminoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=32)</td>
<td>(n=72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>19 (59.4)</td>
<td>50 (69.4)</td>
<td>0.217</td>
</tr>
<tr>
<td>Stage II</td>
<td>10 (31.2)</td>
<td>16 (22.2)</td>
<td>0.228</td>
</tr>
<tr>
<td>Stage III</td>
<td>3 (9.4)</td>
<td>6 (8.4)</td>
<td>0.563</td>
</tr>
<tr>
<td><strong>Non-seminoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=36)</td>
<td>(n=76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>17 (47.2)</td>
<td>31 (40.8)</td>
<td>0.329</td>
</tr>
<tr>
<td>Stage II</td>
<td>14 (38.9)</td>
<td>18 (23.7)</td>
<td>0.042</td>
</tr>
<tr>
<td>Stage III</td>
<td>5 (13.9)</td>
<td>27 (35.5)</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Military DU is used to improve the armor-piercing features of bullets and missiles and to upgrade armor-plating ability of military vehicles (36-38). Intakes of $^{92}$U exceeding United States Environmental Protection Agency (EPA) standards can lead to increased cancer risk (36-38).

Particularly interesting are the reports by the United Nations Environment Programme (UNEP) of post-conflict DU assessment in Kosovo (39), Serbia and Montenegro (40) and Bosnia and Herzegovina (41) published in 2001, 2002 and 2003, respectively. In Kosovo, soil and water samples were collected from eleven sites where DU had reportedly been used, but the analyses showed low levels of radioactivity (39). In Serbia and Montenegro, two years after the conflict, DU was again detected in soil samples and from bioindicators like lichen (40). However, the levels were extremely low and no significant level of radioactivity was measured (40). In Bosnia and Herzegovina 14 sites were investigated and DU was found in soil, drinking water and air samples at three locations, even after more than seven years post-conflict, albeit at extremely low levels (41). Further studies were recommended by UNEP because of the scientific uncertainties on the long-term behaviour of DU in the natural environment (41).

Jergovic et al. conducted a study (42) on respondents from the area of the Eastern Croatia. The observed respondents had higher than normal amounts of warfare metals in serum, urine and hair, due to shelling and other uses of weapons. Those metals were deposited in the soil and then contaminated food and water. The amounts differed between respondents from areas of heavy and moderate fighting. The upscaled (43) results revealed that most vulnerable were those who were younger than 14 years during the war and war veterans.

To confirm that the findings of $^{92}$U and other metals in the Eastern Croatian soil samples are exclusively associated with the war, it is necessary to repeat the soil analysis several times in other regions in Croatia and nearby states as well. Therefore, attempts to try and determine if the usage of DU ammunition is directly linked with higher cancer morbidity in selected population were not successful. The influence of Croatian War of Independence and Chernobyl disaster on change in the incidence and clinical presentation of some cancer types, such as breast and thyroid cancer, has already been documented (44,45). Previous research has confirmed the change in histological presentation and shift towards advanced stages for thyroid cancer (44) and more invasive lobular breast carcinoma in female Croatian population (45). Changes were present in both post-war years as in years following Chernobyl. It is important to emphasize the positive relation of thyroid cancer incidence and radiation exposure.

It would be too bold of a statement, nor was our research set out to directly link DU with the observed rise in TGCC incidence and stage presentation for our population. With so many unanswered questions like long-term behaviour of DU in the environment, the effect of small doses of radiation and timing of our studied population exposure, one could only propose the possible causative potential of DU and other radioactive elements in the genesis of TGCC. Having in mind the varying TGCC incidence between different populations and thus possibly different birth-cohort effects on its genesis, we assume that the aforementioned events which severely affected our population could be responsible for the observed rise in TGCC incidence.

In conclusion, war-related stress, radiation and possibly DU exposure could lead to testicular malignancies after prolonged latency. However, exposure assessment, biomonitoring and field studies must be performed in order to evaluate true long-term environmental behaviour of DU and impact on human health.

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TRANSPARENCY DECLARATION

Competing interest: none to declare
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31. Saradžević et al. Testis cancer incidence Eastern Croatia
SAŽETAK

Cilj Istražiti moguću povezanost između kontaminacije radioaktivnim i toksičnim elementima, prvenstveno osiromašenim uranom (DU) izvorišta iz černobilske katastrofe, Domovinskog rata u Hrvatskoj i rata u Bosni i Hercegovini, te povećane incidencije tumora zametnih stanica testisa (TGCC) u populaciji istočne Hrvatske.


Rezultati U poslijeratnom je razdoblju u odnosu na prijeratno i ratno, incidencija neseminoma (godišnje 4.5 bolesnika na prema 1.3), seminoma (4.2 na prema 1.2) i sveukupno TGCC (8.7 na prema 2.5) bila je 3.5 puta veća, te su neseminomski tumori bili prezentirani u više uznapredovalom stadiju III (35.5% na prema 13.9%, p=0.013).

Zaključak Korištenje osiromašenog urana u oružanim sukobima, nakon nepoznatog vremena latencije, moglo bi dovesti do razvoja TGCC-a. Kako bi se dokazala moguća uzročna veza između izlaganja osiromašenom uranu i razvoja tumora zametnih stanica testisa potrebna je analiza izloženosti navedenom čimbeniku.

Ključne riječi: neoplazme testisa, nuklearno ratovanje, uran, černobilska katastrofa, Hrvatska