ECRONICON OPEN ACCESS

EC PULMONOLOGY AND RESPIRATORY MEDICINE

Editorial

Cytological Double Test for Lung Cancer Screening

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Received: December 14, 2017; **Published:** January 08, 2018

It is well known that the most common cause of lung cancer deaths is cigarette smoking, although lung cancer has occurred in people who have never smoked. In this case, the most common causes of lung cancer are high levels of air pollution, radon gas, family history, radiation therapy to the lungs [1]. In our recent publication the importance of nuclear wars as well as peacetime nuclear disasters in terms of exposome-related health disturbances has been discussed [2-5]. Ammunition with depleted uranium (DU) penetrators has been used in numerous military actions since 1990. After the bombing with DU projectiles, widespread air pollution due to the release of radioactive particles, both in the explosion and in later years due to corrosion of missiles, could be expected. Depleted uranium has been repeatedly used by the military, approximately every four years since 1991 (Iraq 1991, Bosnia 1994 - 1995, Kosovo, Serbia and Montenegro 1999, Afghanistan 2001 - 2003, Iraq 2003 - 2011), which has induced the low dose radiation (air pollution easily transferable to the remote distances from the place of explosion), slow doses (DU ammunition remnants can be fully oxidized into corrosion products twenty-five to thirty-five years after impact) [6] and its further prolonged contribution to the maintenance of alpha particles radiation [7].

One 120 mm DU tank round impacting against a hard target will create about 950 grams of DU dust, while one burst of 30 mm shells fired by an A-10 aircraft might create 960 grams. In some cases, the amount of dust created may be much higher. When a DU shell hits a hard target DU burns. About 20% of DU vaporizes into a fine dust that can travel long distances and be inhaled by people in the immediate vicinity (up to 400 meters) or up to thousands of miles away [8]. Gatti and Montanari (2004) report that combustion processes create a form of particulate pollution that can be released into the environment [9]. The size of the particles inversely relates to the temperature of the process. In the case of DU, the temperature is higher than 3,000°C. Consequently, inorganic micro- and nano-particles have been generated that could pollute the environment after the explosion of DU missiles. Half the total mass of the uranium oxide consists of particles smaller than the wavelength of visible light [10]. Busby and Morgan (2006) tried to answer the question whether the use of uranium weapons in the Second Gulf War resulted in contamination in Europe. The authors found an excess of uranium in the air along the trajectories across Europe, of some 500 nBq/m^3, assuming that uranium particles originated from the Persian Gulf battlefields. It was found about 48,000 particles of 0.25 μm diameter in one cubic meter. By the authors' approximate estimation, each person would have inhaled about 23 million particles of uranium in six weeks. DU armaments have repeatedly been used during the last 25 years: in Iraq (1991), Afghanistan (2001 - 2003), Iraq (2003), in numerous present conflicts in North Africa or West Asia, which are just the regions where dust storms usually occur [10]. As well as infective agents [11], micro DU particles may be transferred along sand to distant regions of the world [4,5].

DU exerts mixed, radioactive (α -, β -, γ- emitter's) and chemical toxicity of a heavy metal on the biosystem. Knowledge concerning uranium or DU toxicity has evolved since 1999 when DU was considered as a Group III agent (not classifiable as carcinogenic to humans) by the International Agency for Research on Cancer (IARC). According to Baverstock (2006), DU has been categorized as a Group I agent – alpha emitter (i.e. as carcinogenic to humans) [12]. The use of DU or release of toxic and ionizing substances during peacetime nuclear disasters induces hormetic effects on the environment, as well as on living organisms [7].

Inhalation is a dominant pathway of internal contamination by DU. Given the half-time of tissue bioavailability of 1,470 days which is expected in the case of inhalation of uranium oxides, as Durakovic reported in 1999 [13], a wide range of clinical manifestations can occur, depending on the individual predispositions of the exposed persons [3,4]. As military campaigns in which nuclear weaponry was used were repeated in the territories which were geographically close to each other almost every 1470 days (approximately 4 years), there is a possibility that every person in a contaminated area has been exposed for a whole lifespan to low slow repeated doses of ionizing radiation from internally deposed particles of DU or other inhaled alpha-emitting radionuclides.

The time interval between the formation of a nonmalignant tissue lesion and its transformation into a malignant one is the most important for planning of further clinical follow-up and therapeutic approaches [5].

Depleted uranium particles can be found in the circulation, or in tissues, obtaining higher penetration power if they are micro- or nano- sized. Uranium is an emitter of alpha radiation, regardless whether it originates from natural sources, peace-time nuclear disasters or military use of several thousand tons of DU. Alpha-particles induce ionization in the environment, as well as in living tissues [7].

Studies on early and delayed health effects of DU contribute to a better understanding of the interaction of DU with a living mater, as a potential internal source of low slow doses of ionizing radiation. Given the extremely long half-life of uranium radionuclides from DU (billions of years), our subsequent studies have to be oriented towards the living world and man in the contaminated environment. Some recent publications from 2013 onwards [2-5] discussed the environmental and health effects of DU. Some longstanding paradigms in the field of radiobiology of DU were changed. Medical entities, as Balkan's or Gulf War Syndrome, are subsumed into a common entity whose pathogenesis is based on radiobiology of the time-dependent effects of DU in military personnel, as well as exposed civilians. Discussion on the early and delayed health effects of depleted uranium is based on authentic medical observation of differential cell counts of bronchoalveolar lavage (BAL) samples from 225 pediatric patients. All the patients, whose bronchoalveolar lavage samples were analyzed, originated from the territories which were geographically close to each other (Serbia and Montenegro seaside and Bosnia and Herzegovina, the territories of the former Yugoslavia that were repetitively stroked by DU armaments) [2,3,5]. The presence and a significant increase in frequency of the Lupus Erythematosus Cells (LEC) in native bronchoalveolar lavage (BAL) detected with the increased yield after the NATO bombing of Serbia, enabled search for the relationship between the presence of LEC in BAL and DU originated alpha radiation. "Unusually" high succumb of LEC in individual BAL specimens were found in the first 6 months after the air strikes against targets in Serbia. The results presented clearly implicate time-dependent effects of DU [2,3]. The LEC phenomenon in BAL has been highlighted as an early radioadaptive tissue response and one of the main, although non-specific characteristic of DU induced low-dose radiation alveolitis.

The discussion on delayed health effects of DU reconnects known facts about smoking exposure and lung cancer (Zunic., *et al*. 2007) with existing low-slow-radiation-doses from tobacco smoke as well as from environmental radioactive air-pollutants. It is not possible to exclude the fact that all patients (smokers and non-smokers alike) were exposed to low-dose DU radiation originating from inhaled pollutants after the bombing of FRY, or the Persian Gulf, according to our publications (Zunic, 2013 [2]; Zunic, 2013-1 [3]; Zunic and Rakic, 2015 [4]). Non-smokers are considered as exposed only to environmental doses.

Smoking exposure values do not provide enough information regarding lung cancer risk among smokers. The predictive value of micronuclei for cancer risk is well documented [14]. With the aim of proposing a lung cancer screening method by using cytological and cytochemical analysis of BAL specimens, we observed the utility of smoking exposure values (pack-years) and apoptotic parameters including apoptotic clearance by alveolar macrophages (AM) [15], and micronucleus test assay (MN) [16]. Lung cancer screening prolongs survival.

The chest radiograph is a routine procedure, easy to perform, and well accepted by patients, but it is not sensitive enough as computerized tomography (CT) scan. The data from CT screening for lung cancer has proven to be debatable in terms of costs and benefits of this widely used procedure. According to Midthun and Jett (2009) [17], who summarized a prevalence of nodules and cancers detected in a prospective study, based on the results of ten studies involving spiral CT screening, out of 53,399 subjects included, 628 cases of lung cancers were detected, out of which 510.25 in surgical stage I A/B. The use of CT scan as a screening tool is highly rational! Nevertheless,

in a recent study, Mathews and coworkers (2013) [18] explained that overall cancer incidence was 24% greater for exposed than for unexposed people. The mean length of follow-up was 9.5 years after exposure.

Cigarette smoke is the main cause of lung cancer, which affects the lungs' defense system at the morphological and cellular level. According to Martel's study (1983) [19] carcinogenic effect of tobacco smoke may act synergically with radionuclides in tobacco, mostly alpha-emitters which induce cumulative doses at bifurcations. Besides tobacco, radioactive particles may be inhaled from environmental sources, as it is air-pollution, including depleted uranium. After inhalation, radioactive particles may be embedded into the tissue (Figure 1a). Depleted uranium particles are mixed, alpha, beta, gamma emitters, and chemical intoxicant of a heavy metal. Embedded particles maintain continuous very-low-slow doses of irradiation of the surrounding tissue structures, inducing cell death, mutation, or repair. Alveolar macrophages are actively involved in the process of apoptotic bodies removal, which are generated from non-resident immunocytes, recruited "on demand" from blood into the lungs [5].

There is no difference in the pathogenesis of cancerous lesion in the lungs which may be developed as a result of smoking habit, or due to internal contamination with radionuclides from the environment or from tobacco. Early tissue response is a nonmalignant lesion which precedes delayed cancer lesions [5]. The time interval between the formation of a nonmalignant tissue lesion and its transformation into a malignant one is the most important for planning of further clinical follow-up and therapeutic approaches. Smoking exposure values are significantly different among smokers and nonsmokers, but they do not provide enough information regarding lung cancer risk among smokers. The use of biopsy for MN assessment from single sites may be misleading as a marker of carcinogen exposure or as an estimate of cancer risk. The analysis of BAL specimens may overcome this problem. Methodological approaches can be different, related to staining technique or the criteria for defining an MN. The May-Grunwald stain followed by Giemsa stain is suitable for differential cell count of BAL, as well as for MN test assay. The literature data showed that there was no an overall increase in smokers' MN frequency, whereas heavy smokers showed a significant increase in genotoxic damage as measured by the MN test assay in lymphocytes [20]. This bimodal answer confirms our explanation of the hormetic-threshold biodosimetric model [5]. We proposed a clinical nomogram based on apoptotic parameters as well as smoking exposure for further testing so as to determine whether every new examinee is at risk by belonging to one of the groups, representing healthy nonsmokers, control smokers and patients with lung cancer.

The graph depicted in figure 1b can be used as a clinical nomogram suitable for further testing so as to determine whether every new examinee is at risk by belonging to one of the groups, representing healthy non-smokers, control smokers and patients with lung cancer. In dose-response relationship (Figure 1b), the dose (along x line) represents smoking and low/slow-dose-radiation exposure. Response (along y line) represents the response of tissue and reflects the complex network of a cell to cell interactions and tissue remodeling, including the balance between apoptosis and apoptotic cell clearance. The functional relationship between apoptotic parameters is presented by a neural network method [21]. The graph depicted in figure 1b shows the field of adaptive tissue response in smokers (H1), the field which represents exhausted adaptive mechanisms (H2) and corresponds to the possible premalignant lesion. Owing to decreased ability of alveolar macrophages to remove apoptotic bodies from tissue, a malignant lesion develops (C).

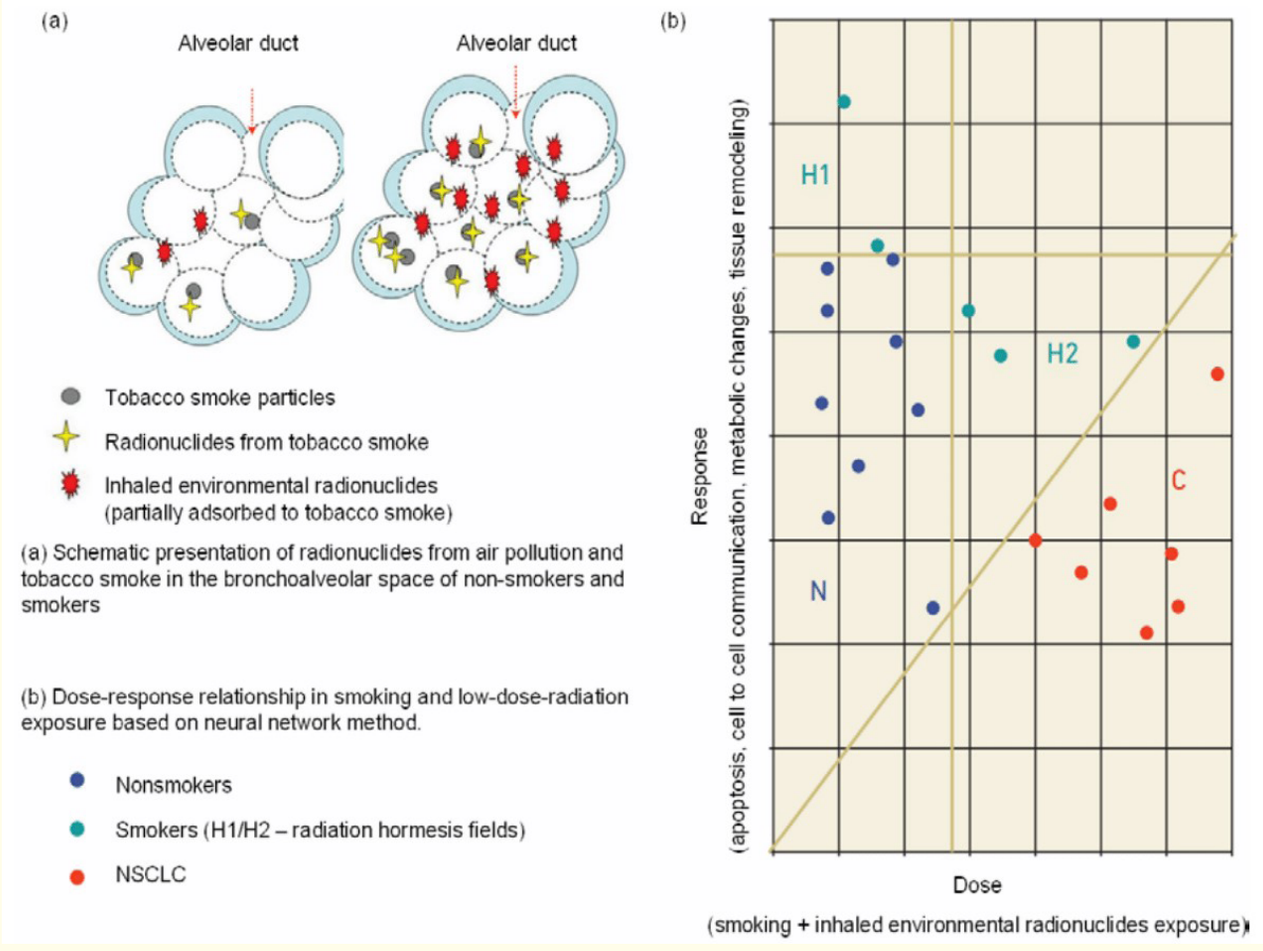

Figure 1: *A clinical nomogram for further testing so as to determine whether every new examinee is at risk by belonging to one of the groups, representing healthy non-smokers, control smokers and patients with lung cancer [5].*

There is a possibility of combining the models of tissue response to low dose radiation. This nomogram represents the hormesis-threshold model of low dose-response to ionizing radiation from internal sources, mostly alpha emitters, based on the neural network method [5].

In our pilot study, all patients with lung cancer were heavy smokers with a smoking history of 30 or more pack-years. This is the reason why we believe that all smokers who undergo bronchology unit should be included in the fast screening procedure that we presented as an initial step in clinical examination. Simplicity and low cost are advantages of this method [5].

Once internalized by the cell, metal-oxide particles exert the cytotoxic effect that is inversely proportional to the particle size, but the presence of DU particle in the tissue is not obligatory to understand that the person was exposed. There is the lack of an appropriate method for exact measuring of internal doses. The recognizing and quantification of biological changes at a cellular or molecular levels in the living media may be the more appropriate procedure for evaluation of low-dose radiation, than measurement by dosimetry techniques [9].

This manuscript raises the possibility of combining the models of tissue response to genotoxic influences. It is possible to use the double test including apoptosis and smoking exposure dose-response relationship and the results of MN test assay to detect the transition of a premalignant lesion into a malignant one in bronchoalveolar space with a high level of accuracy and precision.

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Volume 7 Issue 2 February 2018