METAL AND CARBON-BASED PARTICLES / CLUSTERS IN THE PERIPHERAL T - CELL LYMPHOMA IN THE LUNGS

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Abstract

Submicron particles occurring in the environment are of natural origin, but are also formed by anthropogenic activities as secondary products. The source of these submicron particles are e.g. energy industries, ore processing, cosmetic and pharmaceutical industries, smoking, transportation, etc. Chemical composition of these particles from single sources is not precisely known. Due to their size (<1 µm) they can be easily inhaled and thus enter deep respiratory tract into the lungs. Depending on their size and chemical composition they may have the ability to induce oxidative stress in cells, which lead to damage to the body eventually. The paper addresses occurrence of unspecified peripheral T-cell lymphoma in a 74-year-old male patient, non-smoker, previously working in metal rollers worker. This case was evaluated from the pathological point of view, together with a detection of submicron particles/clusters in the sample of the tumor tissue. Histologically the tumor was in lung tissue inaccurately bounded with diffusely growing medium sized to large lymphoid elements having nuclei of irregular size and shape and with one or multiple nucleoli with prominent mitotic activity. Dark pigment was also dispersed in the tissue during examination by light microscopy at high magnification under polarized light and also foreign particles probably of metallic character were observed. Experimental method used included scanning electron microscopy with energy dispersive spectroscopy (SEM - EDX) and Raman microspectroscopy. SEM - EDX revealed presence of particles/clusters in sizes <500 nm based mainly on iron. Occurrence of iron particles namely siderite ($FeCO_3$) was confirmed by Raman microspectroscopy. Moreover the tumor tissue was identified to contain carbon black and graphite-based particles.

Keywords: Submicron metal and carbon-based particles/clusters, T-cell lymphoma, scanning electron microscopy, Raman microspectroscopy

1. INTRODUCTION

Iron is abundant universally and during evolutionary processes iron became an oxygen carrier in the human body. However, iron works as a double-edged sword, and its excess is a risk for cancer, presumably via generation of reactive oxygen species [1]. They consist of cells of different sizes, some with distinctive accompanying nonmalignant infiltration (B-lymphocytes, neutrophils and others) [2,3].

The task of the metal rollers worker is revisal and service of equipment for rolling metallic materials for the production of various preparations. The main part of the work is straightening profiles, sheets and pipes, grinding and burning surface defects of rolled materials, shapening and dimensional sorting of materials, management of heating ingots and billets, rolling of ferrous and ferrous rolling mills and more. Profession is practiced mainly in rolling mills. Operating workers are exposed t unfavorable working environment including high temperatures and noise, as well as dust and other contaminants [4]. These processes are known to produce particulate emissions released to the air and contributing to occupational exposure [5].

The objective of the study was to detect presence of particles/clusters in tissue of the cancer patient occupationally exposed to iron particles. Shape and elemental and phase composition of the detected particles/clusters will be analyzed. 2.

2. MATERIALS AND METHODS

This study deals with the 74-year-old male patient, non-smoker, who was diagnosed with peripheral T cell lymphoma unspecified in lung tissue which belongs to a heterogeneous group of T lymphomas not included to any clearly defined categories. He previously worked as a metal rollers worker.

2.1. Sample preparation

Tissue samples were fixed in 10 % formalin, dehydrated in 96% alcohol and embedded in paraffin in Tissue Tek VIP automat (Sakura). Subsequently, paraffin blocks were used for tissue fixation. Then samples were cut on 2-4 µm thin slices, which were placed on a glass slide and subsequently dewaxed by xylene and alcohol 96 % and 70%. Tissue sections were dried and used for further analyses.

2.2. Methods used

Raman spectroscopy in combination with a confocal microscope (XploRA™, Horiba Jobin Yvon) was used as non-destructive method to obtain the information about the phase composition of detected particles in the tissue. Laser with a wavelength of 532 nm and a grating 1200T was used for the analysis. In addition, to analyze individual points, the Raman spectral map of the selected area was also performed. This analysis covers the entire selected area and distance of the points of the spectral map apart is chosen individually, depending on the measured sample/area. For a more precise characterization of morphology and determination of the elemental composition of the detected solid particles scanning electron microscope with energy dispersive spectrometer (Philips XL30 and Quanta FEG 450 (FEI) with EDX analysis APOLLO X (EDAX)) was used. Samples were sputter coated with thin film of gold and palladium to acquire electric conductivity.

3. RESULTS AND DISCUSSION

This case was evaluated from the pathological point of view, together with a detection and phase analysis of submicron particles/clusters in the sample of the tumor tissue. Histologically the tumor was in lung tissue inaccurately bounded with diffusely growing medium sized to large lymphoid elements having nuclei of irregular size and shape and with one or multiple nucleoli with prominent mitotic activity. Dark pigment was also dispersed in the tissue during examination by light microscopy at high magnification under polarized light and also foreign particles probably of metallic character were observed. To evaluate the size and shape of the particles/aggregates detected in the tissue samples using Raman microspectrometry, mapping was performed in the selected area. This method has the advantage compared to measurements at individual points, that the entire chosen area is analyzed.

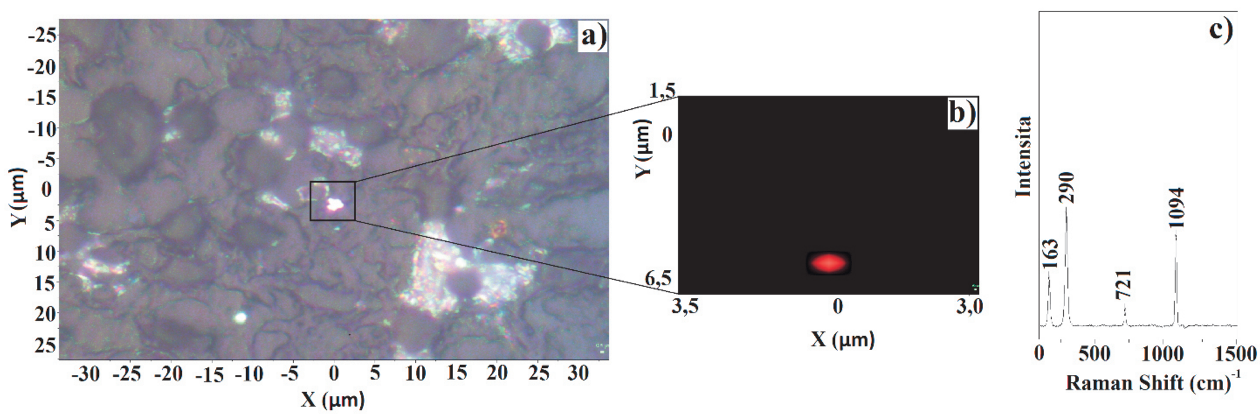


Fig. 1 Raman spectral map of the selected area of the lung cancer tissue, where a) light microscopy image of the tissue with the selected area, b) the spectral map of the selected area (red - siderite, black - surrounding tissue) and c) the measured Raman spectrum of siderite

Fig. 1a shows a sample of the lung cancer tissue which was taken using a light microscope, which is included in the XploRA™ system. According to this picture particle/aggregate of the size of about 3 µm was found. When spot analysis was performed it was found that the particle/cluster consists of siderite (**Fig. 1c**). However, when mapping of the entire selected area was carried out it was found that the particle dimension is below 1 µm in fact (**Fig. 1b**). Another compound detected in lung tumor tissue using Raman microspectroscopy was amorphous carbon (carbon black) (**Fig. 2c**). The carbon black detected under the light microscope had a particle size in the range of 10- 20 µm (**Fig. 2a**). The resulting Raman (red) map shows the size and real shape of particle/cluster of amorphous carbon (**Fig. 2b**). This method of analysis across selected areas of a sample for the above purposes appears to be promising and helpful for closer understanding of real size and shape of particles/clusters present in human tissues analyzed. This tool can significantly contribute to the overall characterization of particles present in tissues.

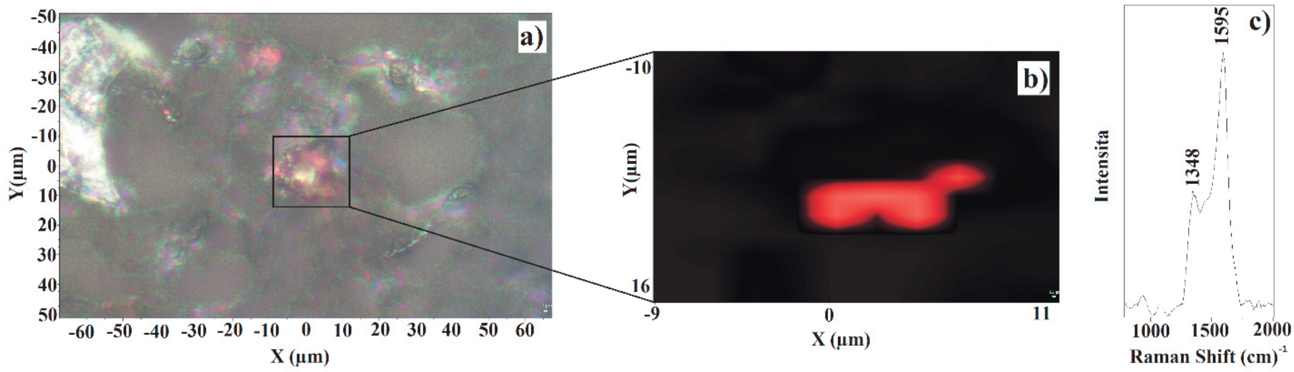


Fig. 2 Raman spectral map of the selected area of the lung cancer tissue, where a) light microscopy image of the tissue with the selected area, b) the spectral map of the selected area (red - carbon black, black - surrounding tissue) and c) the measured Raman spectrum of carbon black

To better clarify morphology and elemental composition of the detected particles in samples of lung cancer tissues SEM - EDX was used. SEM - EDX revealed particle size below 1 µm (**Fig. 3**) or a cluster of particles (**Fig. 4**).

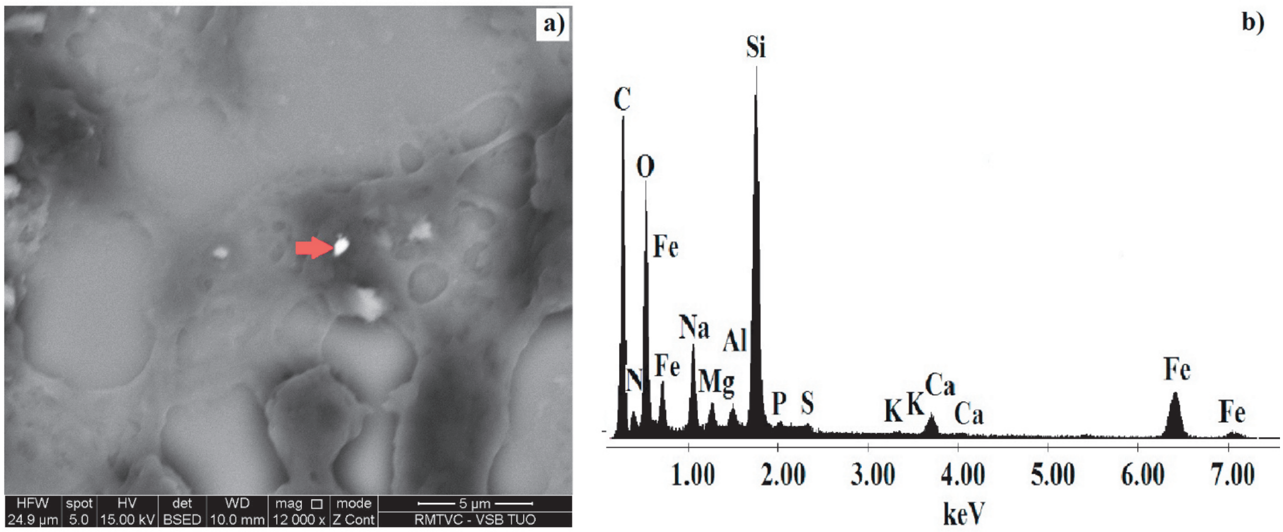
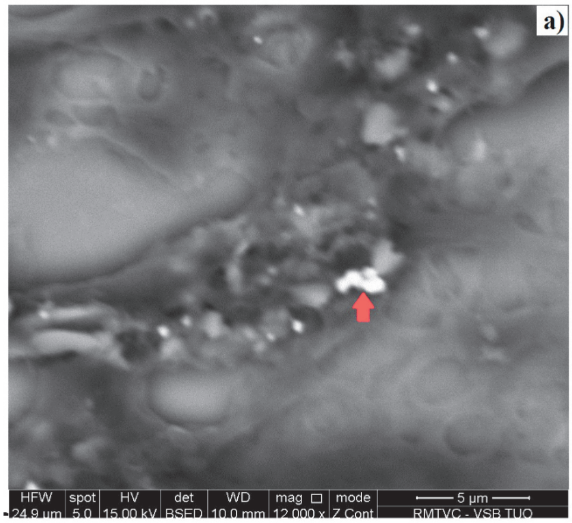


Fig. 3 SEM image of particle containing iron detected in the lung tumor tissue sample (a) with the corresponding EDX spectrum (b)



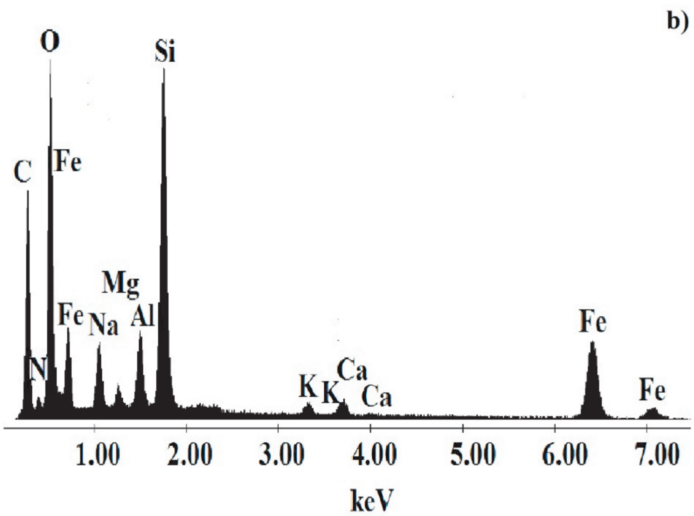


Fig. 4 SEM image of a cluster of particles containing iron detected in the lung tumor tissue sample (a) with the corresponding EDX spectrum (b)

The main anthropogenic sources of the emitted particles/clusters containing iron are pyrometallurgy and steelmaking. However, these iron particles could be also generated and released into the environment by traffic as well, since iron could be one of the main elements in brake pads [6, 7]. For particles containing iron it is known that after penetration into the body it is likely to increase the production of reactive oxygen species (ROS), which may result in oxidative stress and inflammation [8-10]. Apopa et al. demonstrated that exposure to synthetic iron nanoparticles - maghemite (Fe_2O_3), cause an increase in the permeability of endothelial cells by oxidative stress modulating microtubule [11]. Xu et al. performed case-control study found that lung cancer risk was significantly higher for those who worked 15 or more years in the industry of smelting and rolling [5]. Rodriguez et al. demonstrated that workers who have ever been employed in plants with blast furnaces had increased risk of lung cancer, compared with the reference group of workers who were not employees of the departments of metal production [12]. Obviously, iron oxides are important industrial lung toxic substances and iron and steelmaking is one of the industries that has been classified by International Agency for Research and Cancer (IARC) as implying a carcinogenic risk to humans [13]. According to this case study it cannot be concluded that there is a clear causal relationship between occupational exposure to metal particles, mainly iron compounds, and the formation of the tumors in the lung tissue. However, the results obtained by the analysis of the tumor tissue in the lungs and by many other international studies suggest a possible link between long-term exposure to iron-based particles of the human body and their potential harmful effects.

Presence of iron was confirmed in the tissue sample by SEM - EDX, but no specific compound could be determined. However, by Raman microspectroscopy only one iron compound was detected - siderite. According to the IARC the siderite is not considered to be carcinogenic, but long time exposure and/or accumulation of these particles in the tissues could pose a risk to the health.

4. CONCLUSION

We have shown that in the analyzed tumor sample iron-based particles/clusters below 1µm were found. Particles of these submicron dimensions can induce oxidative stress and adverse effects of healthy tissue, althoughwe cannot concludethat the detected particles are the main cause of the observed pathology. Possible source of the detected iron compound is occupational exposure of the patient. Raman microspectroscopy with SEM - EDX combination represents suitably techniques for the detection and comprehensive characterization of micron and submicron particles in human tissues.

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