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Environmental nanoparticles are significantly over-expressed in acute myeloid leukemia

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ABSTRACT

The increase in the incidence of acute myeloid leukemia (AML) may suggest a possible environmental etiology. PM2.5 was declared by IARC a Class I carcinogen. No report has focused on particulate environmental pollution together with AML. The study investigated the presence and composition of particulate matter in blood with a Scanning Electron Microscope coupled with an Energy Dispersive Spectroscope, a sensor capable of identifying the composition of foreign bodies. 38 peripheral blood samples, 19 AML cases and 19 healthy controls, were analyzed. A significant overload of particulate matter-derived nanoparticles linked or aggregated to blood components was found in AML patients, while almost absent in matched healthy controls. Two-tailed Student's *t*-test, MANOVA and Principal Component Analysis indicated that the total numbers of aggregates and particles were statistically different between cases and controls (MANOVA, $P < 0.001$ and $P = 0.009$ respectively). The particles detected showed to contain highly-reactive, non-biocompatible and non-biodegradable metals; in particular, micro- and nano-sized particles grouped in organic/inorganic clusters, with statistically higher frequency of a subgroup of elements in AML samples. The demonstration, for the first time, of an overload of nanoparticles linked to blood components in AML patients could be the basis for a possible, novel pathogenetic mechanism for AML development.

1. Introduction

Acute myeloid leukemia (AML) is a malignant blood cells disorder that is characterized by blocked or severely impaired differentiation of hemopoietic stem cells, resulting in abnormal accumulation of immature precursors and suppression of growth and maturation of cells sustaining normal hemopoiesis. There is no proven single cause of AML, and a combination of factors appears to play a role, also involving gene-environmental interactions [1]. Until now, researches have been mainly focused on exposures to viruses, previous chemotherapy, as well as radiations, chemicals or, in general, some occupational hazards [2–4].

As a matter of fact, a number of chemicals of the most different origin have long been suspected to be leukemogenic. Because of the numerous substances with known myelotoxic activities, it is reasonable to assume that anything capable of injuring the hematopoietic cells might also favor the in-site development of a neoplasm. Apart from benzene, sporadic reports describe AML following exposure to pesticides and insecticides, weed killers, industrial chemicals, shoe-making products, hair dyes and tobacco smoke, but the cause-effect relationship, if any, of most of these agents has yet to be epidemiologically demonstrated [5,6].

The environmental and indoor pollution are due to the emissions of industries, incineration plants, vehicular traffic, and household heating and cooking. The fine Particulate Matter (PM) is a heterogeneous mixture of solid particles and liquid droplets found in the air, differing in size, morphology and chemical composition that can express different physicochemical and toxicological properties in humans and environment. PM can vary in size from particles a-few-tens-of-μm large down to ultra-fine particles with a diameter ≤ 100 nm (nano-particles), and different PM sizes can

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take different pathological pathways independently. Their composition varies according to the different sources of origin; minerals and metals such as iron and its alloys, lead, copper – some of them highly reactive to oxygen (ROS, Reactive Oxygen Species) – and biological substances [7] are generally described. It was previously shown that, due to its tiny size, PM can enter the human body, either through inhalation/respiration or ingestion, gaining free access to the blood [8]. The final destination of this PM, as well as its interactions with circulating blood elements or solid tissues, is impossible to guess *a priori*. It has recently been documented, by means of a new technique combining Environmental Scanning Electron Microscopy (ESEM) and the x-ray microprobe of an Energy-Dispersion Spectroscope (EDS), that nanoparticles can be found in the blood or trapped in solid tissues [9,10]. This physical technique, well known by physicists for the study of materials and nanomaterials, was applied to the biological samples for the first time within the European Project Nanopathology (FP5-QOL-2002-05, 146).

Metabolic interactions with key pathways in cellular survival and transformation should not be excluded, suggesting a possible new area of studies on pathogenesis.

In fact, often, environmental nanoparticles are not biodegradable and, once in the blood and carried by its stream, may be selectively captured by different tissues without any apparent possibility of elimination. The selective uptake can depend either on their size and on their chemical composition or both. Their permanence in the blood could activate the immune-system. In fact, in-vitro nanotoxicological studies [11] have shown that the exposure of human endothelial cells to SiO_2 or Ni nanoparticles has a pro-inflammatory effect by release of IL-8, and that they cause chronic inflammatory reactions over time that could turn to cancer. Now, the effect of the environment on the epigenetic asset of live beings is being increasingly appreciated [12,13]. Epigenetic modulation occurs during the entire lifespan, and the exposure to environmental chemicals can disrupt the epigenetic programming [14,15] besides increasing cancer risk [16]. In this regard, some reports indicated that PM-containing environmental contaminants (e.g., nickel, chromium) contribute to deregulated histone acetylation, therefore favoring epigenetic changes [17]. As a consequence, the presence of PM in the blood or in solid tissue could be responsible for the deregulation of several immunologic or genetic/epigenetic pathways, that could finally turn to cancer. On the other hand, the presence of nanoparticles in cell nuclei [9], as previously observed, may induce to postulate a possible stochastic interference of nanoparticles with the DNA, particularly in the cells during their replication phases [18]. The hypothesis grows more and more credible especially if we consider that many of the nanoparticles containing heavy metals are also chemically hyperreactive [19,20]. We, then, analyzed the blood of a series of AML patients, compared to a control group, applying Environmental Scanning Electron Microscopy (ESEM) and Energy Dispersive Spectroscopy (EDS) methodologies, aiming at identifying the possible presence of micro-, sub-micron and nano-sized foreign bodies in the blood, their location and chemical composition.

2. Patients and methods

2.1. Study population

The study was performed between April 2013 and January 2015. Briefly, peripheral blood samples were obtained from 38 subjects, 19 with a diagnosis of AML according to the World Health Organization, and 19 healthy volunteers (controls recruited from the local population) with an age range 25–65, with no smoking behavior and similar height and weight. AML blood samples were collected at diagnosis. The characteristics of AML patients are listed in Table 1. Signed written informed consent was obtained before enrolment from both patients and healthy volunteers. The study was approved by an independent research Ethics Committee and was done in accordance with the International Conference on Harmonization of Good Clinical Practice Guidelines, the Declaration of Helsinki (1996), and local regulatory requirements and laws.

Table 1
Characteristics of AML patients.

Median age (range)	65 (20-71)
Sex	
Male	12
Female	7
FAB subtype	
M0	1
M1	10
M2	4
M4	2
M5	1
Not evaluable	1
Karyotype	
Favorable	1
Intermediate	8
Unfavorable	7
Not evaluable	3
De novo / secondary AML	11/8

2.2. Sample preparation

The blood samples were centrifuged immediately after the taking at 2000 rpm for 10 min at 25 °C without any anti-clotting agents. Three distinct phases were obtained: the upper layer (plasma, containing clotting factors and platelets), the middle layer ('buffy coat'; white blood cells), and the bottom layer (red blood cells) [21]. In order to avoid any contamination of the plasma with the underlying buffy coat and red blood cell layers, differential centrifugations [22] were performed to allow a better split of the platelets and the plasma fraction. Then, withdrawn buffy-coat phases were lysed with ammonium-chloride buffered solution [23], whereas erythrocyte phases were collected and directly diluted in sterile phosphate buffered saline.

After washing and fixation with 2.5% glutaraldehyde in 0.1 M saline solution for one hour, 20 μl of the suspension was deposited on acetate supports as previously described [23] and placed in a moist and sealed chamber at 4 °C. Every blood fraction was verified by means of Flow Cytometry (BD FACSCalibur™, USA).

The slides were then washed and, after an alcohol ascending-concentration dehydration, were critical-point dried. They were then mounted on aluminum SEM stubs and observed under a Field Emission Gun Environmental Scanning Electron Microscope (FEG-ESEM, Quanta 200, FEI, The Netherlands) to identify cell morphologies and the possible presence of foreign bodies thanks to their higher atomic density. The chemical composition of the particles was analyzed through the X-ray microprobe of the Energy Dispersive Spectroscope (EDS, EDAX, Mahwah, NJ, USA) coupled to the instrument. The interaction of the electron beam, emitted by the gun, with the sample induces an excitation, energy is lost and a single X-ray is emitted that is characteristic of the element hit. The use of this type of microscope, thanks to the possibility to work at low vacuum, prevents possible sample contamination and/or the creation of artifacts. The observations were made by means of different detectors (SE: secondary-electron detectors and BSE: backscattered-electron detectors), and performed at medium vacuum, at acceleration voltages varying from 10 to 30 kV to detect the size, morphology and elemental composition of the organic and/or