Leukemia Research 50 (2016) 50–56

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Environmental nanoparticles are significantly over-expressed in acute myeloid leukemia

G. Visani[a,*,1], A. Manti[b], L. Valentini[b], B. Canonico[b], F. Loscocco[a], A. Isidori[a], E. Gabucci[a], P. Gobbi[b], S. Montanari[c], M. Rocchi[c], S. Papa[c], A.M. Gatti[d,1]

[a] Hematology and Stem Cell Transplant Center, AORMN, Pesaro, Italy
[b] Dept. of Earth, Life and Environment Sciences, University of Urbino Carlo Bo, Urbino, Italy
[c] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy
[d] National Council of Research of Italy, Institute for the Science and Technology of Ceramic Materials, Faenza, Italy

ARTICLE INFO

Article history:
Received 24 March 2016
Received in revised form 5 August 2016
Accepted 1 September 2016
Available online 3 September 2016

Keywords:
Acute myeloid leukemia
Nanoparticles
Metals
Oxidation
Environmental pollution
Forensic pathology

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

* Corresponding author at: Giuseppe Visani, MD, Hematology and Hematopoietic Stem Cell Transplant Center, AORMN, Via Lombroso 1, 61122 Pesaro-Italy.
E-mail address: pesarohematology@yahoo.it (G. Visani).
[1] These authors equally contributed to the manuscript.

http://dx.doi.org/10.1016/j.leukres.2016.09.004
0145-2126/

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

Table 2
Number of aggregates and particles in cases and controls.

Samples n.	Cases Aggregates/Particles	Controls Aggregates/Particles
1	7/65	2/17
2	0/0	2/3
3	10/1964	0/0
4	4/288	3/53
5	9/295	2/2
6	4/381	4/4
7	12/495	4/23
8	8/154	1/1
9	9/164	2/2
10	9/177	2/2
11	7/176	2/2
12	11/237	3/34
13	5/45	0/0
14	3/60	3/47
15	1/1	0/2
16	5/71	9/9
17	11/188	6/6
18	8/325	0/0
19	18/308	0/0
Total	141/5394	45/207

Cases (n.19), the total of studied cases; aggregates case (n.141), the total of aggregates in all cases; Particles cases (n. 5394), the total of particles in all cases; controls (n.19), the total of studied controls; aggregates controls (n.45), the total of aggregates in all controls; particles in controls (n. 207), the total of particles in all controls.The appropriate *t*-test was performed to test significance of differences of means between cases and controls: t, statistic value; df, degree of freedom; P value, result of *t*-test . MANOVA, the multivariate analysis of variance, corroborated the result from the *t*-tests.*p value refers to MANOVA applied to aggregates and particles simultaneously analyzed.

inorganic particles identified. Single particles (with dimensions down to 10 nm) or organic-inorganic aggregates were identified, evaluated and counted. The chemical spectra of the particles were analyzed and the frequency of every element was counted.

2.3. Data analysis

Every ESEM image collected was analyzed for the presence of foreign bodies (white dots in the figures) in a double blind fashion, and the counting was performed manually since the GSR automatic software developed by FEI Company to count "bodies" with size bigger than 50 nm did not recognize clearly the aggregates and made mistakes. The data were analyzed using the Statistical Package for Social Sciences version 20 (SPSS, Chicago, USA). Unpaired two-tailed Student's *t*-test and the multivariate analysis of variance, MANOVA, were used to compare the aggregates and single particles between cases and controls. Unpaired two-tailed Student's *t*-test and Principal Component Analysis were performed for elements, as univariate and multivariate analysis.

All data are represented as total number of particles; statistic test (t), degree of freedom (df) and result of *t*-test or MANOVA test were indicated. Two-sided tests were used in all calculations. Significance level was fixed at $\alpha = 0.05$ for all the statistical analyses.

3. Results

The general characteristics of the particles identified in the samples obtained from AML patients or healthy controls are shown in Table 2. A large quantity of micron-, submicron- and nano-sized foreign bodies (from 20 μm down to 100 nm) was documented in 18/19 AML cases, whereas they were absent or rare in the controls. Single particles were detected in close contact with blood elements (Fig. 1a,b) or entrapped in blood proteins, so forming composite organic/inorganic aggregates, with a size ranging from 5 to 20 μm. (Fig. 1c,d). A total of 141 aggregates (median 8, range 0–18) in AML, compared to a total of 12 aggregates in controls (median 1, range 0–3) were counted. This kind of analysis showed variable sizes and variable number of particles per aggregate, with a total of 5394 particles in AML cases compared to a total of 207 in controls. Table 2 shows the number and distribution of aggregates and particles in AML samples and controls. The number of foreign bodies was statistically different (MANOVA, $P < 0.001$ and $P = 0.009$, Table 3). Also, Principal Component Analysis clearly pointed out a strong difference between cases and controls, showing an evident over-dispersion of the former group along both the first and the second Principal Component (Fig. 3).

The aggregates were then analyzed with Energy Dispersive Spectroscope, a sensor capable of identifying the composition of nano-particles, identifying their elemental composition by means of the chemical spectrum obtained (see Fig. 1c). Being they an organic-inorganic mixture, elements like C, O, Na, Cl, K, P, naturally present in the biological tissues and in the saline used in the sample processing, were not considered in this analysis.

The aggregates resulted rich in heavy metals such as iron, chromium, nickel (i.e. the alloy of stainless steel), titanium and lead. The frequency of the elements in the particles identified in the blood of cases and controls are listed in Table 4. All the elements were more frequent in pathological cases as compared to controls (Fig. 2). Si, Al, Fe, Ti, and Cu were significantly more frequent in AML ($P = 0.03$, $P = 0.03$, $P = 0.002$, $P = 0.04$, $P = 0.02$ respectively). Ni (101 vs 5), Cr (142 vs 65), and Pb (57 vs 2) were clearly over-represented in the AML subgroup, even if not reaching statistical significance, probably due to the small sample size ($P = 0.124$, $P = 0.328$, $P = 0.155$, respectively).

4. Discussion

Acute myeloid leukemia (AML) accounts for approximately 25% of all leukemia cases in adults in the Western World, with the highest incidence in the US, Australia and Western Europe [24]. Continuing increases in the incidence of leukemia since the late 1970s may suggest a possible environmental etiology correlated to the general increase of the environmental pollution and following PM [25].

Recently, the International Agency for Research on Cancer (IARC, Lyon, France) confirmed that there is sufficient evidence of an association of leukemogenesis with exposure to benzene and 1,3 butadiene. Moreover, in 2013, the same agency declared that $PM_{2.5}$ is a Class I carcinogenic agent. So, it looks reasonable to presume that not only benzene and its derivatives, but also other components of $PM_{2.5}$, such as black carbon (BC), aluminum (Al), calcium (Ca), iron (Fe), lead (Pb), sulphur (S), silicon (Si) and titanium (Ti) could have a role in the pathogenesis of AML. Furthermore, epigenetic features like DNA methylation, which is profoundly altered in AML cells, are increasingly being implicated as sensitive to environmental exposures [26]. In fact, DNA methylation modifications, both in specific genes and in non coding repetitive elements, have been shown to be altered following PM exposure [27].

This paper demonstrates for the first time the significant overload of particulate matter-derived nanoparticles linked to blood components in AML patients. Particulate matter-derived nanoparticles were almost absent in matched healthy controls. These nanoparticles frequently consisted of highly reactive elements such as Si, Al, Fe, Ti, and Cu, and were significantly over represented in AML samples with respect to healthy controls ($P = 0.03$, $P = 0.03$, $P = 0.002$, $P = 0.04$, $P = 0.02$ respectively). The PM-derived nanoparticles found in AML samples showed different chemical compositions that varied from stainless steel (iron-chromium-nickel alloy) to iron-sulphur, bismuth-phosphorus, antimony-cobalt, aluminum-copper or to very unusual alloys containing lead. Furthermore, there were particles with never-previously-documented composition, namely, materi-

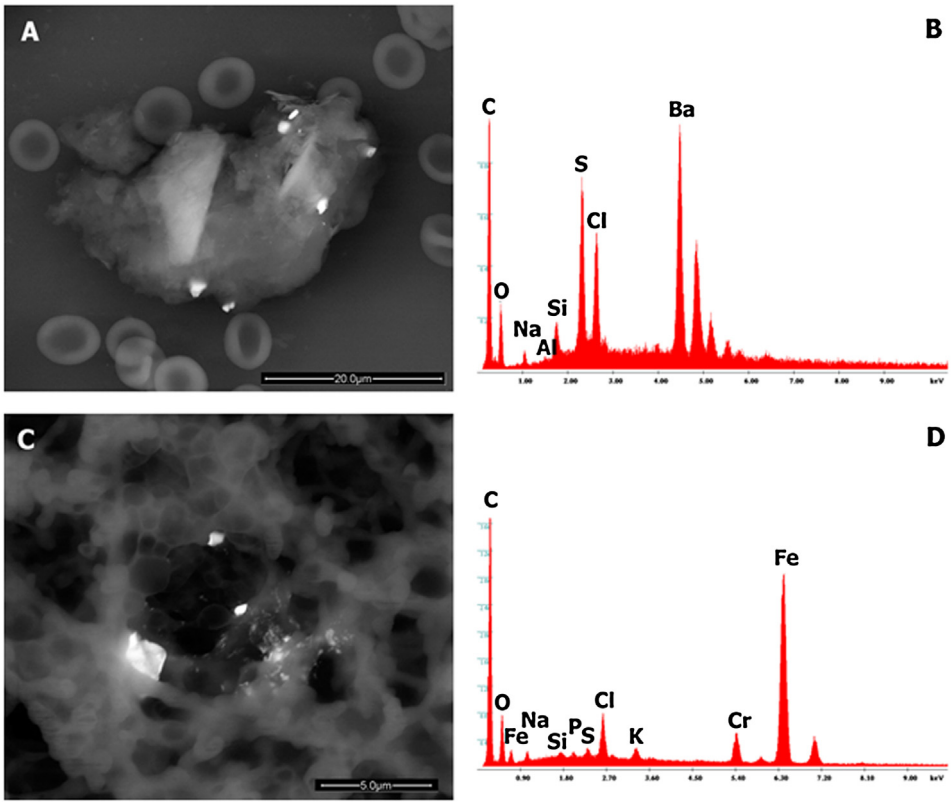

Fig. 1. Examples of an aggregate of micro- and nanosized particles embedded in a biological blood reactive tissue (A). The particles (whiter than the biological substrate for the higher atomic density) entrapped are composed of barium-sulphur-chlorine-silicon (B). Single submicronic and nanosized particles are showed in C. They are composed of iron-chromium-nickel, i.e. stainless steel (D).

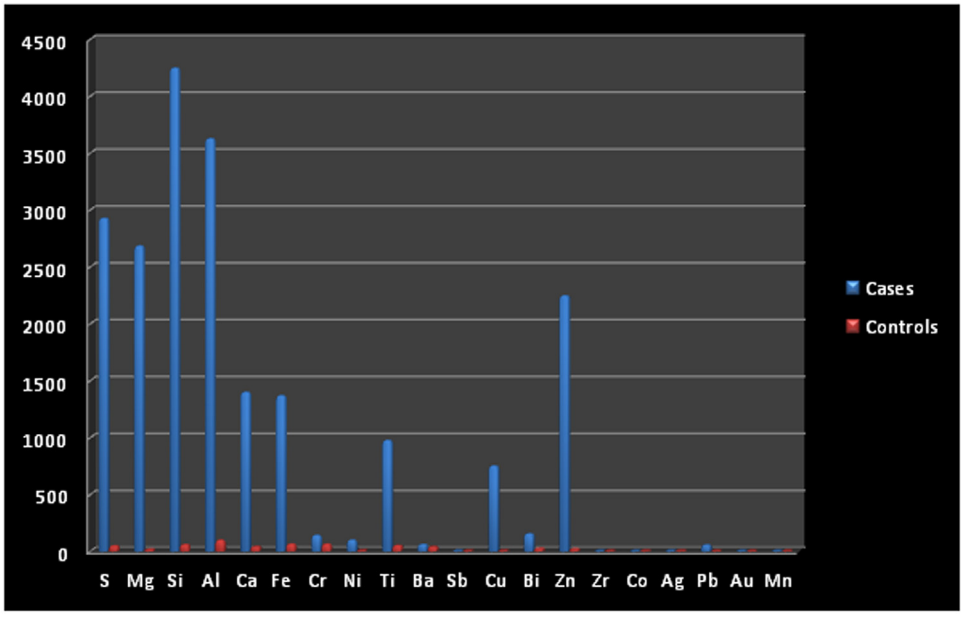

Fig. 2. Elements present in the particle composition identified in cases and controls.

Table 3
Test of significant differences between aggregates and particles in cases and controls.

	Independent samples T-test			MANOVA
	t	df	P *value*	P *value*
Aggregates	4,565	27,654	<0,001	<0,001*
Particles	2,750	18,065	0,013	0,009

The appropriate *t*-test was performed to test significance of differences of means between cases and controls: *t*, statistic value; df, degree of freedom; P *value*, result of *t*-test . MANOVA, the multivariate analysis of variance, corroborated the result from the *t*-tests.

* p value refers to MANOVA applied to aggregates and particles simultaneously analyzed.

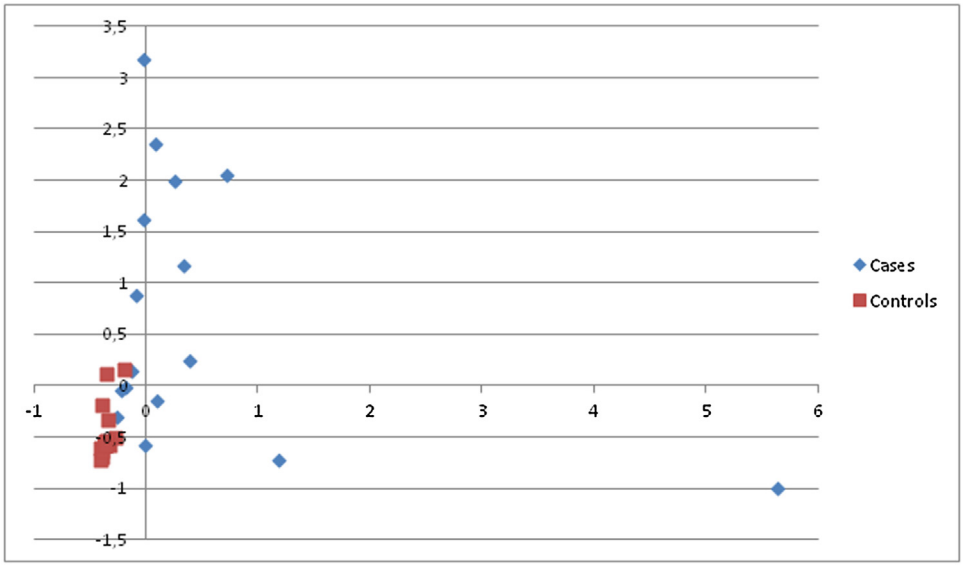

Fig. 3. Graph of principal component analysis of elements. The first (x-axis) and the second (y-axis) Principal Components (PC) resulted associated with Mg, S, Si, Al, Zn (First PC) and Ca, Fe, Cr, Ni, Ba, Co, Au (Second PC). The first and the second PC accounts for the 33.83% and 12.88% of the total variance, respectively.

Table 4
Comparison between the elements found in cases and controls.

Elements	Cases N	Controls N	Independent samples T-test t	df	P *value**
Significantly different					
Si	4249	60	2.226	18.027	0.039
Al	3628	100	2.361	18.047	0.030
Ca	1399	42	3.633	18.121	0.002
Fe	1369	66	3.596	18.565	0.002
Ti	977	52	2.206	18.540	0.040
Cu	752	4	2.546	18.002	0.020
Statistical trend					
S	2929	49	1.881	18.015	0.076
Mg	2686	16	1.907	18.001	0.073
Not significantly different					
Cr	142	65	0.992	36	0.328
Ni	101	5	1.615	18.040	0.124
Ba	62	41	0.396	36	0.695
Zn	2248	21	1.227	18.004	0.236
Zr	1	2	−0.588	36	0.553
Co	1	0	1.000	18.000	0.331
Ag	3	0	1.484	18.000	0.187
Pb	57	2	1.484	18.105	0.155
Au	1	1	0.000	36	1.000
Mn	1	0	1.000	18.000	0.331
Bi	156	25	1.051	18.938	0.307

Elements, elements found in the particles that make up the aggregates observed; Cases N, total number of each elements in cases; Controls N, total number of each elements in controls; *t*, statistic value; df, degree of freedom; P-value, result of *t*-test.

als not included in any handbook of materials like, for instance, an alloy containing carbon-aluminum-oxygen-bismuth-clhorine-sulphur-calcium-potassium-silicon-sodium-barium-copper. For it, an origin from a casual, not controlled combustion, for instance one from a waste incineration plant, is suspected because of the spherical morphology of the particle and the abnormal quantity of elements (up to a total of 16) present in its EDS spectrum.

Interestingly, nanoparticles found in AML samples were shown to be linked to plasmatic as well as to blood cellular components, forming aggregates, with peculiarities in shape, composition as well as spatial location. A statistically significant larger number of aggregates was found in AML patients with respect to normal controls (141 vs 12). Moreover, when we looked at the sizes and number of particles per aggregate, we found a total of 5394 particles in AML cases compared to a total of 207 in controls. Finally, aggregates found in AML patients were significantly enriched in Si, Al, Fe, Ti, and Cu.

We then searched for the possible leukemogenic mechanism played by the nanoparticles aggregates, and we observed a close contact between the aggregates and blood cells. In addition, we detected interactions between the aggregates of highly reactive elements and plasma proteins and coating by protein, something that may be due to a protein "corona" formation [28,29] induced by the physical-chemical properties of the particles (size, morphology, chemical composition, electrical charges, surface free energy, etc.). In fact, when a submicron- or nano-sized material is immersed in a physiological environment, it rapidly adsorbs proteins forming a layer known as the protein "corona" [30], which can alter the surface properties of the material, giving origin to a new three-dimensional structure that can dynamically interact with the

biological environment (e.g: blood). This new composite macromolecule can trigger a physio-pathological response due to the unfolding of the proteins adsorbed that can activate the immune system, change the expression of selected genes, or produce epigenetic modifications. In this regard, several observations produced the proof of principle that $PM_{2.5}$ could be responsible for genetic and epigenetic modifications. First, dust has been demonstrated to be able to change the expression of selected genes in AML, as shown on HL60 cell line with the seasonal meteorological phenomenon carried by the wind known as Asian Dust (AD), i.e. clouds of particles composed mainly of silicon combined with other elements [31]. This is confirmed by the ability of urban dust to interfere at gene level in leukemic cells growth and survival. Second, previous investigations have suggested that DNA methylation is highly sensitive to environmental exposure, including air pollution [32–36]. More recently, PM exposure was demonstrated to be able to generate reactive oxygen species (ROS), which are chemically reactive molecules that can induce oxidation of 5-methylcitosine (5mC) to 5-hydroxymethylcitosine (5-hmC), that is significantly enriched at gene transcription start sites coinciding with TET1 on genes with high CpG-content. Furthermore, in contrast to 5mC which is often associated with suppressed gene expression, 5hmCis specifically enriched in expressed genes and could thus play a major role in activating and or maintaining gene expression, leading to the development of AML. Third, myeloid precursors produce large amounts of enzymes that can both generate cytotoxic mediators as part of their normal function (e.g. myeloperoxidase), but can also activate environmental chemicals able to interact with cells, producing genotoxic intermediates [37], linked to the formation of chromosome rearrangements typical of leukemia, such as benzene and its derivatives [38,39].

In conclusion, the overload of $PM_{2.5}$-derived submicronic and nanoparticles linked to blood components observed only in samples taken from AML patients could be responsible for the increased genomic instability in myeloid precursors, which is the basis for AML development. Furthermore, the generation of the protein "corona", that can dynamically interact with the biological environment, could contribute to the activation of the immune system, the change in the expression of selected genes, or the production of epigenetic modifications, thus contributing to the transformation of normal precursor into AML blasts. Last but not least, it is important to underline that, even if we can presume that PM could be somehow responsible for the development of AML, this is absolutely not certain because a sure link has not yet conclusively been found.

5. Conclusions

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. To discover the exact mechanisms of interaction further evaluation and the planning of dedicated genetic and epigenetic correlative studies are needed. Next generation sequencing analyses, that are discovering new genes and pathways in leukemia [40], and linking up to population-based epidemiologically-derived data on exposures to professional, dietary or environmental factors, will probably be able to clarify the role of nanoparticles in the pathogenesis of AML. Anyway, these ultrastructural pathological findings open the door to new disciplines like forensic pathology, correlating the presence of internal contamination with the environmental exposure.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Acknowledgements

Authors are indebted to Dr. Federico Capitani, Dr. Guglielmo Ragazzoni and Mrs. Lavinia Nitu for their skillful technical help. Dr. Claudio Pizzagalli, Director of Pesaro Department – Regional Environment Protection Agency of Marche (ARPAM) is also thanked for his strict and friendly supervision of the research work. The study was supported in part by AIL Pesaro Onlus.

References

[1] A. Renneville, C. Roumier, V. Biggio, O. Nibourel, N. Boissel, P. Feaaux, et al., Cooperating gene mutations in acute myeloid leukemia: a review of the literature, Leukemia 22 (2008) 915–931.
[2] J.M. Pogoda, S. Preston-Martin, P.W. Nichols, R.K. Ross, Smoking and risk of acute myeloid leukemia: results from a Los Angeles County case-control study, Am. J. Epidemiol. 155 (2002) 546–553.
[3] G.J.L. Kaspers, U. Creutzig, Pediatric acute myeloid leukemia: international progress and future directions, Leukemia 19 (2005) 2025–2029.
[4] D.P. Sandler, G.W. Collman, Cytogenetic and environmental factors in the etiology of the acute leukemias in adults, Am. J. Epidemiol. 126 (6) (1987) 1017–1032.
[5] F. Lo Coco, C. Nervi, G. Avvisati, F. Mandelli, Acute promyelocytic leukemia: a curable disease, Leukemia 12 (12) (1998) 1866–1880.
[6] A. Mele, M. Szklo, G. Visani, M.A. Stazi, G. Castelli, P. Pasquini, et al., Hair dye use and other risk factors for leukemia and pre-leukemia: a case-control study, Am. J. Epidemiol. 139 (6) (1994) 609–619.
[7] K. Peters, R.E. Unger, A.M. Gatti, E. Sabbioni, R. Tsaryk, C.J. Kirkpatrick, Metallic nanoparticles exhibit paradoxical effects on oxidative stress and pro-inflammatory response in endothelial cells in vitro, Int. J. Immunopathol. Pharmacol. 20 (4) (2007) 685–695.
[8] A. Nemmar, P.H. Hoet, B. Vanquickenborne, D. Dinsdale, M. Thomeer, M.F. Hoylaerts, et al., Passage of inhaled particles into the blood circulation in humans, Circulation 105 (4) (2002) 411–414.
[9] A.M. Gatti, S. Montanari, Nanopathology: the Health Impact of Nanoparticles, Pan Stanford Publishing, Singapore, 2008, pp. 1–298.
[10] A.M. Gatti, S. Montanari, Case Studies in Nanotoxicology and Particle Toxicology, Elsevier Pub, USA, 2015, pp. 1–260.
[11] A.M. Gatti, J. Kirkpatrick, A. Gambarelli, F. Capitani, T. Hansen, R. Eloy, et al., ESEM evaluations of muscle/nanoparticles interface in a rat model, J. Mater. Sci. Mater. Med. 19 (4) (2008) 1515–1522.
[12] R.R. Kanherkar, N. Bathia-Dey, A.B. Csoka, Epigenetics across the human lifespan, Front. Cell. Dev. Biol. 2 (2014), article 49.
[13] S.A. Tammen, S. Friso, S.-W. Choi, Epigenetics: the link between nature and nurture, Mol. Asp. Med. 34 (4) (2013) 753–764.
[14] A.J. Bernal, R.L. Jirtle, Epigenomic disruption: the effects of early developmental exposures, Birth Defects Res. Part A: Clin. Mol. Teratol. 88 (10) (2010) 753–764.
[15] T. Schoofs, W.E. Berdel, C. Müller-Tidow, Origins of aberrant DNA methylation in acute myeloid leukemia, Leukemia 28 (2014) 1–14.
[16] M. Romani, M.P. Pistillo, B. Banelli, Environmental epigenetics: crossroad between public health, lifestyle, and cancer prevention, BioMed. Res. Inter. 587983 (2015) 1–13.
[17] C. Liu, J. Xu, Y. Chen, X. Guo, Y. Zheng, Q. Wang, et al., Characterization of genome-wide H3K27ac profiles reveals a distinct $PM_{2.5}$-associated histone modification signature, Environ. Health 14 (65) (2015) 1–8.
[18] A.M. Gatti, D. Quaglino, G.L. Sighinolfi, A morphological approach to monitor the nanoparticle-cell interaction, Int. J. Imaging 2 (2009) 2–21, S09 Spring-Editorial 1.
[19] A. Takagi, A. Hirose, T. Nishimura, N. Fukumori, A. Ogata, N. Ohashi, S. Kitajima, J. Kanno, Induction of mesothelioma in p53+/− mouse by intraperitoneal application of multi-wall carbon nanotube, J. Toxic. Sci. 33 (1February) (2008) 105–116.
[20] B. Trouiller, R. Reliene, A. Westbrook, P. Solaimani, R. Schiest, Titanium dioxide nanoparticles induce DNA damage and genetic instability In vivo in mice, Cancer Res. 69 (November 15) (2009) 8784.
[21] D.P.S. Sengar, F.N. Jerome, R.J. Douglas, A simple method for separation of buffy coat from peripheral blood of chickens, Can. J. Comp. Med. 32 (4) (1968) 593–597.
[22] P.H. Carter, S. Resto-Ruiz, G.C. Washington, S. Ethridge, A. Palini, R. Vogt, et al., Flow cytometric analysis of whole blood lysis, three anticoagulants, and five cell preparations, Cytometry 13 (1992) 68–74.
[23] B. Canonico, M. Betti, F. Luchetti, Flow cytometric profiles, biomolecular and morphological aspects of transfixed leukocytes and red cells, Clin. Cytometry 78B (4) (2010) 267–278.
[24] H. Dohner, D.J. Weisdorf, C.D. Bloomfield, Acute myeloid leukemia, N. Engl. J. Med. 373 (12) (2015) 1136–1152.

[25] F. Han, Y. Tan, W. Cui, L. Dong, W. Li, Novel insights into etiologies of leukemia: a HuGE review and meta-analysis of CYP1A1 polymorphisms and leukemia risk, Am. J. Epidemiol. 178 (4) (2013) 493–507.
[26] V.J. Cogliano, R. Baan, K. Straif, Y. Grosse, B. Lauby-Secretan, F. El Ghissassi, et al., Preventable exposures associated with human cancers, J. Natl. Cancer Inst. 103 (24) (2011) 1827–1839.
[27] M. Sanchez-Guerra, Y. Zheng, C. Osorio-Yanez, J. Zhong, Y. Chervona, S. Wang, et al., Effects of particulate matter exposure on blood 5-hydroxymethylation: results from the Beijing truck driver air pollution study, Epigenetics 10 (7) (2015) 633–642.
[28] M. Lundqvist, I. Sethson, B.H. Jonsson, Protein adsorption onto silica nanoparticles: conformational changes depend on the particles' curvature and the protein stability, Langmuir 20 (2004) 10639–10647.
[29] Z. Deng, G. Mortimer, T. Schiller, A. Musumeci, D. Martin, R. Minchin, Differential plasma protein binding to metal oxide nanoparticles, Nanotechnology 20 (2009) 45510.
[30] S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. Kuharev, A. Rekik, et al., Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis, ACS Nano 5 (9) (2011) 7155–7167.
[31] Y.J. Choi, H.Q. Yin, E.J. Park, K. Park, D.S. Kim, B.H. Lee, Gene expression analysis of so called asian dust extracts in human acute myeloid leukemia cells, Toxicol. Res. 26 (1) (2010) 21–28.
[32] V. Bollati, A. Baccarelli, Environmental epigenetics, Heredity 105 (2010) 105–112.
[33] V.K. Cortessis, D.C. Thomas, A.J. Levine, C.V. Breton, T.M. Mack, K.D. Siegmund, et al., Environmental epigenetics: prospects for studying epigenetic mediation of exposure-response relationships, Hum. Genet. 131 (2012) 1565–1589.
[34] L. Hou, X. Zhang, D. Wang, A. Baccarelli, Environmental chemical exposures and human epigenetics, Int. J. Epidemiol. 41 (2012) 79–105.
[35] J.A. Alegria-Torres, A. Baccarelli, V. Bollati, Epigenetics and lifestyle, Epigenomics 3 (2011) 267–277, PMID:22122337.
[36] A. Baccarelli, S. Ghosh, Environmental exposures, epigenetics and cardiovascular disease, Curr. Opin. Clin. Nutr. Metab. Care 15 (2012) 323–329.
[37] P. Lonkar, P.C. Dedon, Reactive species and DNA damage in chronic inflammation: reconciling chemical mechanisms and biological fates, Int. J. Cancer 128 (9) (2011) 1999–2009.
[38] Q. Lan, L. Zhang, G. Li, R. Vermeulen, R.S. Weinberg, M. Dosemeci, et al., Hematotoxicity in workers exposed to low levels of benzene, Science 306 (5702) (2004) 1774–1776.
[39] L.1 Zhang, N. Rothman, Y. Wang, R.B. Hayes, G. Li, M. Dosemeci, et al., Increased aneusomy and long arm deletion of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene, Carcinogenesis 19 (11) (1998) 1955–1961.
[40] Cancer Genome Atlas Research Network, Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia [published correction appears in N Engl J Med 369(1): 98], N. Engl. J. Med. 368 (22) (2013) 2059–2074.