A rapid method for assessing the accumulation of nanoplastics in human peripheral blood

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INTRODUCTION

Plastic pollution is a global problem. Animals and humans can ingest and inhale plastic particles, with uncertain health consequences. Potential effects of nanoplastic exposure may be associated with alterations in the xenobiotic metabolism, nutrients absorption, energy metabolism, cytotoxicity, and behavior. In humans, no data on nanoplastic absorptions has been reported previously. Nanoplastics (NPs) are particles ranging from 1 nm to 100-1000 nm that result from the erosion or breakage of larger plastic debris and can be highly polydisperse in physical properties and heterogeneous in composition. Given that its detection is significantly reliant on environmental exposure, we have prospectively studied the presence of NPs in human peripheral blood (PB). Specifically, we have used fluorescence techniques and nanocytometry together with the staining of the lipophilic dye Nile Red (NR), to demonstrate that NPs can be accurately detected using flow cytometry.

MATERIALS AND METHODS

EDTA-anticoagulated peripheral blood samples were obtained from healthy donors and patients from the Hospital Germans Trias i Pujol (HGTiP). Nile Red, a red phenoxazone dye that binds to the surface of plastics and neutral lipids, was used to determine the presence in blood of the most common plastics: low density polyethylene, polystyrene, polyethylene terephthalate, and polyamide. NR staining was performed after the organic matter degradation by incubating 20µL of PB in 1% KOH (1mL final volume) at 60ºC in a dry block for a minimum of 10 days. 200, 500 and 800 nm calibration microspheres (Bangs Laboratories, Inc.) were used on the Attune™ NxT Flow Cytometer (Thermo Fisher), with the H-pulse parameter and collecting the violet-side scattering using the Attune NxT Violet Side-Scatter Filter Kit. In order to avoid potential environment contaminations, NPs measurements were always done in triplicate and a quadruplicate was added when one of the results had discrepancy. Graphs were generated with Prism software (v.9).

The IncuCyte® SX5 Live-Cell Analysis Instrument (Sartorius Stedim) was used as a real-time system to study the phagocytic activity up to two days in a cell culture incubator. Fluorescent green microspheres (Count Check Beads green, Sysmex) were added for the rapid and accurate detection and quantification of in vitro phagocytosis.

RESULTS AND DISCUSSION

Our preliminary results on a sample of about two hundred humans, including healthy blood donors (HD, n=37), newborns (NB, n=21), and patients with acute lymphoblastic leukemia (ALL, n=22), acute myeloid leukemia (AML, n=40), chronic lymphocytic leukemia (CLL, n=11), multiple myeloma (MM, n=28), non-small cell lung cancer (LC, n=15), idiopathic nephrotic syndrome (INS, n=9), COVID-19 (CoV, n=4), and type 1 diabetes (T1D, n=5), confirmed the presence of NPs in PB. Deionized water, KOH 1% and K3-EDTA were also tested to assess the potential traces of NPs in suspension and compared with PB samples (**Figure 1**). LC and CoV had the lowest NP levels detected, whereas ALL and newborns, the highest. Healthy donors had an important dispersion in the NPs measurements, regarding the median value. NB had similar levels when compared with HD, whereas NB provided more homogeneous values and lower dispersion (**Figure 2**). Regarding age, younger individuals provided a slightly higher NP values when compared with elderly individuals (**Figure** 3). In the case of sex, no remarkable differences were observed (**Figure 4**).

Fluorescence microscopy also confirmed the inability of phagocytes to destroy microbeads (**Figure 5**).

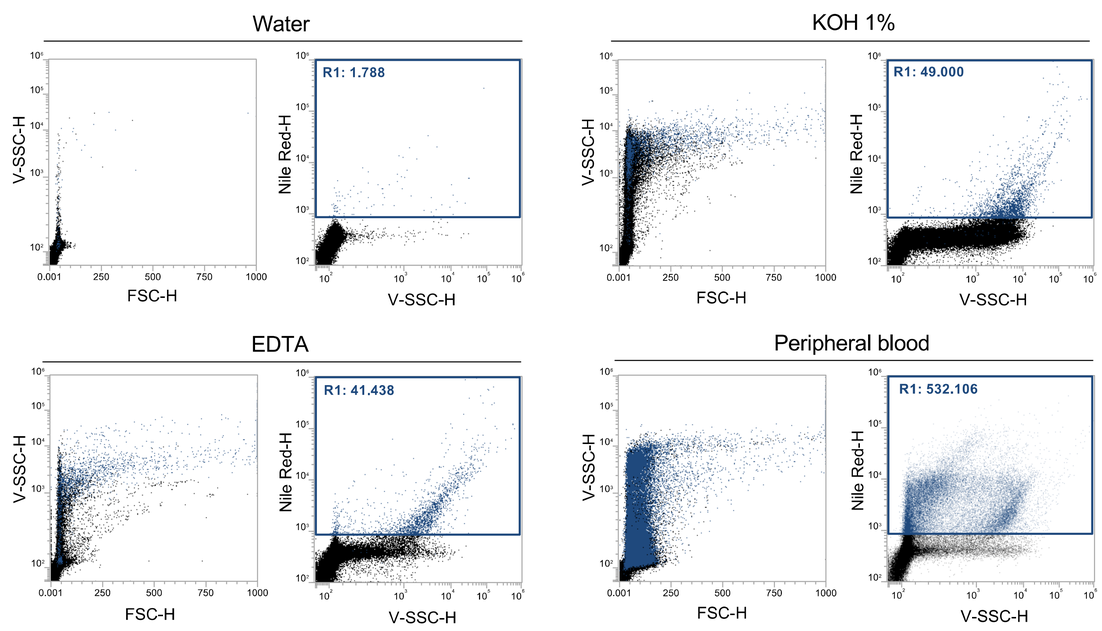

Figure 1. Flow cytometry detection of nanoplastics in peripheral blood. Deionized water, KOH 1%, K3-EDTA, and a representative blood sample are shown. NP concentration (events/µl) is selected in R1. Violet-SSC is displayed in hyperlog scale, FSC in linear scale and Nile Red in logarithmic scale.

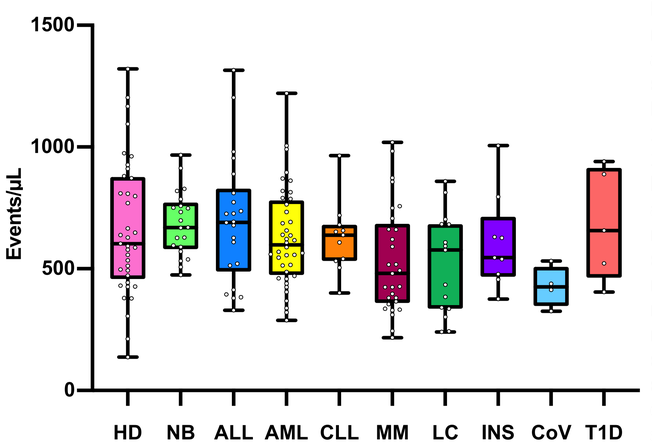

Figure 2. Nanoplastics in healthy donors, newborns and patients with hematological and non-hematological conditions. Median (m) and range (r) values are shown (n=192). Healthy blood donors (HD, n=37, m=610, r=88-1460), newborns (NB, n=21, m=679, r=371-1038), and patients with acute lymphoblastic leukemia (ALL, n=22, m=681, r=188-1384), acute myeloid leukemia (AML, n=40, m=599, r=239-1274), chronic lymphoblastic leukemia (CLL, n=11, m=633, r=331-1001), multiple myeloma (MM, n=28, m=494, r=138-1061), non-small cell lung cancer (LC, n=15, m=511, r=101-963), idiopathic nephrotic syndrome (INS, n=9, m=556, r=354-1077), COVID-19 (CoV, n=4, m=415, r=256-553), and type 1 diabetes (T1D, n=5, m=646, r=377-963) subjects were included.

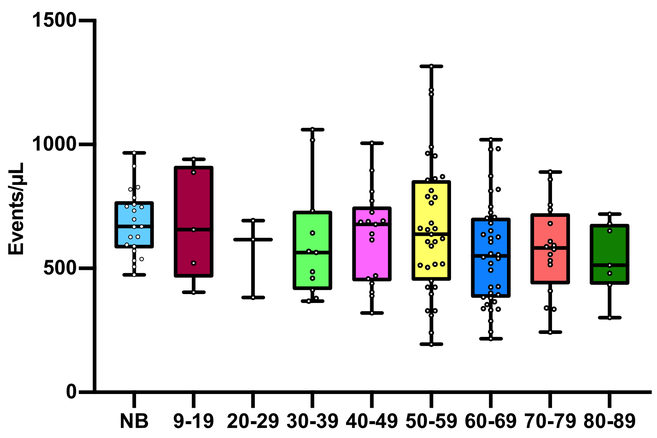

Figure 3. Age-based distribution of nanoplastics in peripheral blood. Age-ranges were classified in decades [median (m) and range (r) are indicated]: 9-19 (n=5, m=662, r=377-963), 20-29 (n=3, m=598, r=347-787), 30-39 (n=11, m=554, r=188-1216), 40-49 (n=17, m=655, r=293-1139), 50-59 (n=35, m=627, r=101-1384), 60-69 (n=36, m=532, r=138-1144), 70-79 (n=16, m=568, r=224-993) and 80-89 (n=7, m=546, r=227-802). Newborns (n=21, m=679, r=371-1038) were characterized by low dispersion measurements.

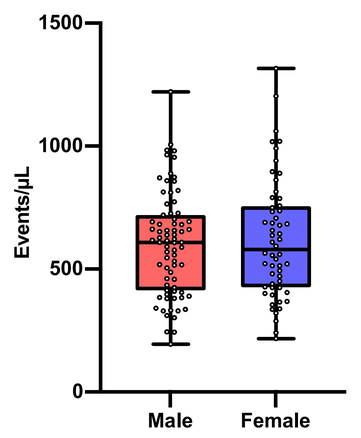

Figure 4. Sex-based distribution of nanoplastics in peripheral blood. A total of n=131 individuals were included in this study: n=75 males (m=603, r=183-1274) and n=56 females (m=598, r=101-1384), where m=median and r=range.

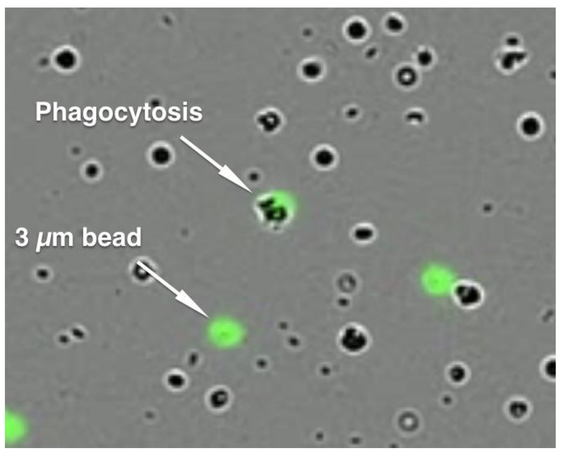

Figure 5. Phagocytes are not able to destroy microbeads. Green fluorescent microspheres were co-incubated with whole blood leukocytes. Phagocytes (12-20µm) were not able to successfully phagocyte the green microspheres (3µm). Image taken with the IncuCyte® SX5 Live-Cell Analysis Instrument (Sartorius Stedim).

CONCLUSIONS

Here, for the first time, we provide a method for detecting NPs in human blood. In fact, this flow cytometry analysis is highly accurate and precise, as well as rapid and simple. Overall, the accumulation of NPs observed in PB may be related to inhalation of NPs originated from wheeled traffic and suspended materials in cities. Further analyses will be needed to associate this accumulation with lifestyle, health and pathology.

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