Supplemental Materials

DETAILED METHODS

Exclusion Criteria

Exclusion criteria were as follows: pregnant/breastfeeding, currently smoking or quit smoking <6 months prior, or smoking history 0-10 years, diagnosed with asthma, inhaled corticosteroid use, regular bronchodilator use, or significant comorbidities considered by the principal investigator to increase cardiovascular risk. In addition, an exercise test was performed to determine VO_2-max, used to set exercise intensity during exposures described below.

Data completeness

One participant did not consent to bronchoscopy and we were not able to obtain blood samples from one participant. Adequate cell counts were required for BAL assays, resulting in the following numbers with complete data: N=17 with blood draws, N=17 blood flow cytometry, N=17 blood smears (N=1 only one exposure), N=17 performed bronchoscopy, N=15 with complete BAL flow cytometry, N=15 with BAL NETs assay (N=1 only one exposure).

Peripheral blood neutrophil flow cytometry

50µl of blood was stained for neutrophil lineage and activation markers (APC-Cy7 CD45, PE-Cy7 CD16, BV421 Activated Mac-1, APC CD66b, BV510 CXCR2, ThermoFisher LIVE/DEAD Red Stain©). Live neutrophils (CD45+/CD16+/CD66b+/LIVE/DEAD-) were identified after excluding debris based on forward scatter, using CD45 APC-Cy7 BD Biosciences Cat #55783, and the following from ThermoFisher: CD16 PE-Cy7, Cat #25-0168-41, CD66b APC Cat #17-0666-41, LIVE/DEAD Red Stain Cat #L23102. Neutrophils were assessed for their expression of CD16, CD66b, Activated Mac-1 (CD11b

Activated BV412, BD Biosciences Cat #566313) and CXCR2 (CD182 BV510 BD Biosciences Cat #744196), quantified as the change from baseline median fluorescence intensity (MFI) across the exposure. An unstained sample was included for every timepoint and used to normalize MFI by subtraction, accounting for slight day-to-day variability. Gates were set using fluorescence minus one controls.

Peripheral blood neutrophil and band cell differential

Blood smears were prepared with 5µl of blood, fixed in ethanol and stained with eosin and methylene blue (Millipore Harleco Hemacolor Stain Set). 500 leukocytes per time point were identified by a skilled observer as mature polymorphonuclear cells (PMN), band cells, eosinophils, or others. For quality control, the percentage of neutrophils counted from blood smears was cross referenced with the complete blood cell differentials (CBCs) obtained from these same samples from Vancouver General Hospital. A coefficient of variance was determined, and referenced against the confidence interval values provide by the Laboratory Quality Assurance Program from the College of Physicians and Surgeons of Saskatchewan (1). Wherever there was disagreement greater than the referenced threshold, a second skilled observer re-counted the blood smears, and the observer count which more closely approximated the hospital CBCs was used for analysis. Our method of band cell counting was chosen as we deemed this more reliable than hospital-ordered counts, the latter of which consists of 200 manually counted cells by a single observer.

BAL neutrophil extracellular traps

150,000 BAL cells were seeded onto uncoated round coverglass for 1h at 5% CO_2 and 37°C. Cells were fixed with 4% paraformaldehyde, blocked (ThermoFisher BlockAid Solution) and stained for hallmark NETs markers, using primary antibodies (anti-H3Cit Rabbit IgG, Abcam Cat #5103; anti-neutrophil

elastase mouse IgG, Dako Cat #M0752), secondary antibodies (Alexa555 goat anti-rabbit IgG, Invitrogen Cat#A21428; Alexa 488 mouse anti-goat IgG, Invitrogen Cat #A228175), and Hoechst 33342 DNA stain (BD Biosciences, Cat #561908). A 4x4 grid of photos for each slide was acquired at 60X magnification and stitched together into one large image (EVOS FL Auto Imaging System). The clearest image for each visit was used to quantify NETs using ImageJ. NETs were defined as structures meeting these three criteria: 1) clear extracellular fibrous citrullinated histone strands, 2) positive DNA stain, and 3) positive NE stain. NET-covered area was quantified by tracing an outline in the overlay image. Then total nuclear area (um^2) was assessed automatically by converting the DAPI channel image to binary with the color threshold tool. NETs were quantified as NET-covered area normalized to total nuclear area and expressed as a % of total nuclear area.

Peripheral blood neutrophil extracellular traps by flow cytometry and immunocytochemistry

$1x10^6$ isolated neutrophils were stimulated for 2h *in vitro* with 100μg/mL DEPs, 4μM ionomycin (positive control) or media alone (negative control). Using a previously described flow cytometry assay, NETs were quantified as the percentage of cells staining triple-positive, without a permeabilization step, for anti-myeloperoxidase (MPO-FITC, Mouse IgG, Abcam Cat #11729), anti-H3Cit (Rabbit IgG, Abcam Cat #5103), APC goat anti-Rabbit IgG (Jackson ImmunoResearch Cat #111-136-144) and DAPI DNA stain (BD Biosciences, Cat #564405). For the immunocytochemistry assay, anti-neutrophil elastase (Mouse IgG, Dako Cat #M0752) was used as a primary antibody, with Alexa488 Goat Anti-Mouse IgG (Invitrogen, Cat #A28175) as the secondary antibody.

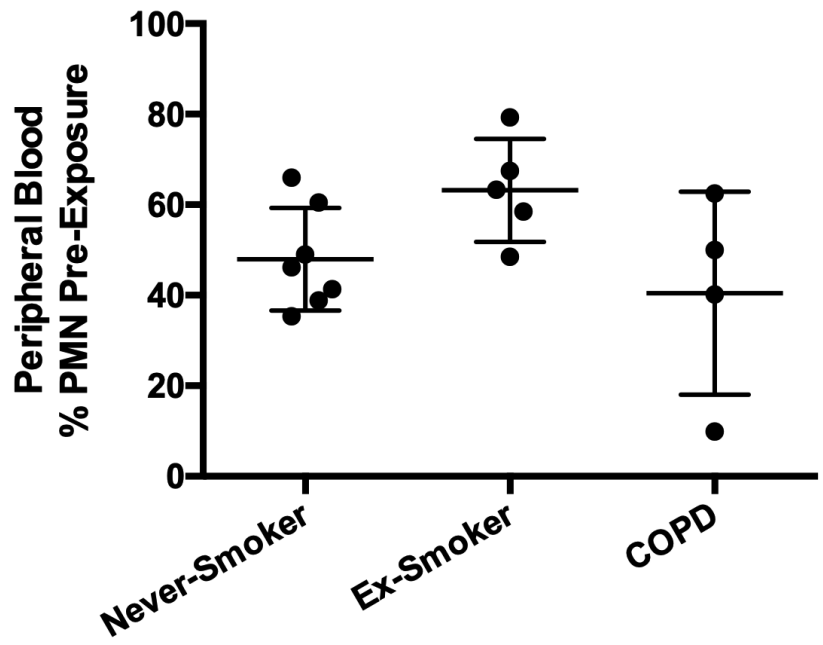

Figure S1. No difference in percentage of baseline polymorphonuclear cells (PMN). Percentage of mature neutrophils from pre-exposure, obtained from blood smear differentials, was compared across groups using repeated measures one-way ANOVA as an indicator of baseline inflammatory state.

REFERENCES

1. College of Physicians and Surgeons of Saskatchewan: Laboratory Quality Assurance Policy Manual. *2016 Edition, p. 39-41*.