Supplementary Material. Analysis of lipid mediators

Selected plasma polyunsaturated fatty acids (PUFAs) and oxylipins (OxLs) were quantified by a targeted LC-MS/MS method following solid phase extraction as previously described [30]. Each batch of analysis includes samples from patients and controls. Briefly, 10 µL of deuterated internal standard mix solution (6-keto PGF1α-d4, LTB4-d4, 5-HETE-d8, and EPA-d5, 125 ng/mL each one) was added to 1 mL of plasma followed by deproteinization with methanol and centrifugation. Lipid mediators were extracted from the supernatant by solid-phase extraction (SPE) using a 10 mg Strata-X SPE cartridge (Phenomenex, Torrance, CA, USA). The eluate was evaporated under a fine stream of nitrogen and the residue was reconstituted in 100 µL methanol. The analysis was performed using an LC-MS/MS system consisting of a Nexera X2 system coupled online to a Shimadzu LCMS-8050 triple quadrupole mass spectrometer equipped with an electrospray ionization source (Shimadzu, Kyoto, Japan). The Nexera system comprises an LC-30AD binary pump, a SIL-30AC autosampler, and a CTO-30A column oven. The injection volume was 5 µL and the autosampler temperature was set at 4°C. The chromatographic separation was performed using a Kinetex C8 analytical column (2.1 mm × 150 mm; 2.6 µm, Phenomenex) with an oven temperature fixed at 40°C. The flow rate was 0.40 mL/min, using 0.1% formic acid/water as solvent A and acetonitrile as solvent B, with a linear gradient. The mass spectrometer was operated under both positive and negative electrospray and multiple reaction monitoring modes. The MS conditions were as follows: nebulizing gas (N2), flow rate: 3.0 L/min; drying gas (N2), flow rate: 10 L/min; heating gas (air), flow rate: 10 L/min; DL temperature: 250°C; block heater temperature: 400°C; interface temperature: 300°C; CID gas (Argon), pressure: 230 kPa. Selected PUFAs and OxLs were identified and quantified by comparing their retention times, MRM coordinates, and peak areas with pre-established data obtained for pure standards. The LabSolutions LC-MS software was used for system control as well as data acquisition and quantification.

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