nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data collection performed with Excel v16.90.2
Data analysis	All statistical analyses were conducted using R v4.3.1 (https://www.r-project.org), and plots were created using Excel.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All raw data are included in the Supplementary Material

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	The study enrolled n=22 controls (10 males, 45%) and n=63 subjects diagnosed with MASLD (n=37 males; 58%). Sex was determined by self-reporting. We investigated whether adding sex as covariates improves the predictive performance of the our model for liver fibrosis with the Likelihood Ratio Test (LRT) and its associated p-value. While sex can be included in the model, it does not appear to be a crucial factor, and the GP model can perform effectively without it. We cannot consider this last result as definitive because of the low presence of females with advanced fibrosis or cirrhosis in our cohort.
Population characteristics	The study accounted for several covariates: age, sex, body mass index (BMI), diabetes, hypertension, dyslipidemia, and medication use, which are outlined in Table 1 of the manuscript.
Recruitment	Individuals (> 18 years) diagnosed with MASLD based on EASL guidelines were enrolled and classified according to the level of fibrosis. Specifically, MASLD was defined by the presence of hepatic steatosis, occurring in subjects with at least one cardiometabolic risk factor and the absence of significant alcohol intake (greater than 20 grams per day for women and 30 grams per day for men). The study was conducted in full conformance with the principles of the Declaration of Helsinki.
Ethics oversight	The study was approved by the local Ethics Committee of the University Hospital Umberto I (reference 6804, 09/11/2022)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The "Integrated phenotyping of the Gut-plAtelet-Liver AXIS in the progression of chronic liver disease" (iGAL-AXIS)" project is an observational, prospective study aimed at exploring the relationship between gut dysbiosis, metabolome composition, inflammation, and platelet activation in chronic liver disease. Based on our preliminary data and considering an average of platelet-leukocyte aggregates (i.e., surrogate markers of platelet pro-inflammatory phenotype) of 22.75 % (SD 18.00) in cirrhotic patients and of 6.59 % in NAFLD patients, we calculated that enrolling 33 subjects per group allows reaching 95% power (α error 0.05). Sample size calculation was performed using the software nQuery Advisor, version 5.0 (Statistical Solutions, Saugus, Massachusetts). In addition, previous studies[19] identified significant differences in platelet transcripts among 21 obese patients before and after bariatric surgery. Thus, we expect that a sample size of n= 33/group should be enough to detect significant changes in the platelet phenotype and pro-inflammatory response. Enrollment is still ongoing and the submitted study is based on the preliminary analysis of part of the cohort we expect to enroll.
Data exclusions	No data were excluded.
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	No randomization was performed.
Blinding	Laboratory personell performed the quantification of inflammatory cytokines and metabolite without prior knowledge of the diagnosis of the patients. Stratification of data was performed after analysis were completed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☐ Antibodies
☐	☐ Eukaryotic cell lines
☐	☐ Palaeontology and archaeology
☐	☐ Animals and other organisms
☐	☒ Clinical data
☐	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☐ ChIP-seq
☐	☒ Flow cytometry
☐	☐ MRI-based neuroimaging

Antibodies

Antibodies used	n/a
Validation	n/a

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	n/a
Authentication	n/a
Mycoplasma contamination	n/a
Commonly misidentified lines (See ICLAC register)	n/a

Palaeontology and Archaeology

Specimen provenance	n/a
Specimen deposition	n/a
Dating methods	n/a

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	the study did not use laboratory animals
Wild animals	n/a
Reporting on sex	n/a
Field-collected samples	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT06623084
Study protocol	https://clinicaltrials.gov/study/NCT06623084
Data collection	Data was collected anonimously
Outcomes	The data presented in the manuscript is a preliminary analysis in a study that is still ongoing and that has the following outcome: To identify platelet features that correlate with liver disease progression.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	n/a
Files in database submission	n/a
Genome browser session (e.g. UCSC)	n/a

Methodology

Replicates	n/a
Sequencing depth	n/a

Antibodies	n/a
Peak calling parameters	n/a
Data quality	n/a
Software	n/a

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Inflammatory cytokines were measured using a multiplex bead-based flow cytometric assay (Biolgend, Inflammation Panel I, catalog number 740809), according to the manufacturer's instructions.
Instrument	Aquisition was performed on a BD Accuri C6 Plus flow cytometer.
Software	C6 Plus Analysis Software for data extraction. Legendplex software for interpolation to a standard curve.
Cell population abundance	A minimum of 6000 events per bead population was acquired.
Gating strategy	Cytokine-specific populations were segregated based on the size and internal APC fluorescence intensity. The concentration of a particular cytokine was quantified based on the PE fluorescent signal according to a standard curve generated in the same assay.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	n/a
Design specifications	n/a
Behavioral performance measures	n/a

Acquisition

Imaging type(s)	n/a
Field strength	n/a
Sequence & imaging parameters	n/a
Area of acquisition	n/a

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	n/a
Normalization	n/a
Normalization template	n/a
Noise and artifact removal	n/a

Volume censoring | n/a

Statistical modeling & inference

Model type and settings | n/a

Effect(s) tested | n/a

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016) | n/a

Correction | n/a

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity | n/a

Graph analysis | n/a

Multivariate modeling and predictive analysis | n/a